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NATIONAL RESEARCH COUNCIL OF CANADA

8422

PROCEEDINGS

OF THE

FIFTEENTH MEETING

OF THE

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

OTTAWA

3 MARCH 1952

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NATIONAL RESEARCH COUNCIL

Proceedings

Fifteenth Meeting

of the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in Council Chamber, National Research Building,
9:30 a.m., Monday, 3 March, 1952.

---

1. Attendance

Dr. J. Stanley Bagnall
Dr. A.C. Burton
Dr. M.H. Garvin
Dr. Arthur W. Ham
Dr. J.D. Hamilton
Dr. L.A. Kilburn
Dr. J.B. MacDonald
Dr. R.A. McDougall
Dr. J.J. Rae
Dr. D.L. Thomson

Mr. S.J. Cook, Secretary

By invitation

Dr. H.A. Hunter
Dr. W.J. Linghorne
Dr. Gordon Nikiforuk
Dr. K.J. Paynter
Dr. O.J. Walker

Apologies for their absence were received from - Dr. R.G. Ellis, Chairman, who was ill with influenza, Dr. H.K. Brown, Dr. C.H. Best, Dean E. Charron, Dr. J.B. Collip, and Dr. H.R. MacLean.

2. Election of Chairman pro tem

It was MOVED by Dr. Ham and SECONDED by Dr. Kilburn,

That Dr. D.L. Thomson be appointed Chairman of this meeting.

CARRIED.

3. Chairman's Remarks

THE CHAIRMAN thanked the members for having chosen him to preside at this meeting. He welcomed Dr. Hunter, Dr. Linghorne, Dr. Nikiforuk,

Dr. Paynter and Dr. Walker, who had come to present reports on their projects in person. He invited them to take part in the discussions on other papers as well as in regard to their own projects.

4. Message to Dr. Ellis

It was MOVED by Dr. McDougall and SECONDED by Dr. Burton,

That a message be sent to Dr. Ellis expressing the regret of the Committee at his absence and conveying best wishes for his speedy recovery.

CARRIED.

5. Minutes of the 14th Meeting

THE CHAIRMAN briefly reviewed the minutes of the 14th meeting.

It was MOVED by Dr. Bagnall and SECONDED by Dr. Ham,

That the Minutes of the 14th Meeting, having been circulated, be taken as read and approved.

CARRIED.

6. Business Arising out of the Minutes

(i) DR 15 (Min. 4 (i), 14th Mtg.)

THE SECRETARY reported that Dr. Norma Ford Walker had written under date 14 February, 1952, to say that Mrs. Hatton, who is holding an appointment as instructor, is re-writing a considerable portion of her manuscript, and will present her thesis this Spring. When it has been completed, and accepted by the graduate school, a copy will be forwarded for inclusion in the Dental Committee records.

(ii) DR 32 (Min. 4 (ii), 14th Mtg.)

THE SECRETARY reported having heard from Dr. Ham as follows:-

"With regard to DR 32 which you may recall only permitted me to engage a part-time worker -- I have already filed two brief progress reports on this project and I had hoped that by the Spring the young man who worked on this project would have been able to have made a complete survey of all his sections and, consequently, to have presented a more complete report. As matters stand, however, although a large number of sections have been cut from the experimental material, there are a number of tooth sections still to be prepared and such material as we have studied to date adds nothing further to the information I have given in the two preliminary reports.

"Accordingly, I do not think we should plan to present anything further at the coming meeting but, in all probability, we should be able to make a complete statement at the subsequent one."

Sept

(iii) Dr. Bagnall's Bibliography on Caries Research (Min. 23, 14th Mtg.)

THE SECRETARY reported that, as directed, he had obtained quotations from the publishers on the cost of reprinting the Bibliography. The quotations were as follows:-

100 copies $2340.
200 copies $2650.
300 copies $2960.

A ballot vote of the Executive resulted in the approval of the purchase of 100 copies at $2340. These volumes have been printed and received.

A statement on the purchase and sale and complimentary distribution of Dr. Bagnall's Bibliography follows:-

PURCHASED:-

209	$ 4,280.00
Tax	342.40
Total	$ 4,622.40
111 volumes (reprinted).....	$ 2,395.00
Tax	239.50
Total	$ 2,634.50
Grand Total	$ 7,256.90

SALES (to 29 February, 1952):-

146 volumes at $10.00	$ 1,460.00
Net cost	$ 5,796.90

In addition to the 146 volumes sold there has been a complimentary distribution of 37 copies.

(iv) Travel To Scientific Meetings (Min. 25, 14th Mtg.)

THE SECRETARY said that notices regarding the availability of travel funds for attendance at the meeting of the International Association for Dental Research had been distributed to all grantees under the Dental Committee. Two applications had been received, notably from Dr. J.J. Rae and Dr. J.B. Macdonald.

These applications had been approved by the Executive and the grantees had been notified.

THE CHAIRMAN said that he understood the sense of the previous meeting to be that provision should be made annually for the attendance of delegates at the meetings of the International Association for Dental Research, and that grantees would accordingly be notified of this provision each year.

Consideration of Reports from Grantees

7. DR 1 - Dr. J.J. Rae

"Chemical Mechanisms in Dental Caries"

DR. RAE presented a summary and interim report No. 13 on his project both of which appear in APPENDIX "H"

DR. RAE added that he was considering preparing a paper for publication on his work which had shown that there was no correlation between l.b.a. counts and lactic acid production; some people think such a correlation actually exists.

8. DR 12 and DR 25 - Dr. A.E.R. Westman

"Investigation of materials for use as cements for methacrylate inlays (DR 12); investigation of methods for the use of methyl methacrylate as direct dental fillings (DR 25)."

THE CHAIRMAN stated that both of these investigations had been terminated and reported to the Committee, but that one additional paper had been received since the last meeting. The memorandum giving the title appears in APPENDIX "B"

9. DR 13 - Dr. M. Doreen Smith

"Fluoride content of enamel and dentin of teeth from Toronto children studied in relation to pablum history of the children's diets."

THE CHAIRMAN presented a report by Dr. Smith which showed that no significant difference has been found between pablum and non-pablum cases in so far as enamel is concerned, but some difference occurs in the dentin. He said that three areas have been investigated: Sarnia, where the water supply has a naturally low fluorine content; Stratford, where the water supply is high in fluorine; and Brantford, where the water supply is naturally low in fluorine and has had fluorine added to it. Dr. Smith was interested in the fluorine content of foods and in a study seeking to determine how far food fluorine, as distinct from fluorine in water supplies, has an effect on teeth.

The summary and report on Dr. Smith's project appear in APPENDIX "N"

DR. WALKER said that the deep-well waters in Alberta are normally high in fluorine whereas surface waters in the province have a low fluorine content.

10. DR 22 - Dr. C.H. Best

"Part I - An investigation of the mechanism of repair of the supporting structures of the teeth;

"Part II - Fusospirochetal infection and pre-existing inflammation."

THE CHAIRMAN stated that two papers had been published and one is in press relating to this project. He then invited Dr. Linghorne to present a report on Part I of this investigation which has been under his charge.

DR. LINGHORNE spoke for an hour outlining the work that he has been doing. The summary and report appear in APPENDIX "I"

THE CHAIRMAN then presented a summary and report by Mr. D.R. O'Connell, who has been working on Part II of this investigation; this summary and report also appear in APPENDIX "I"

DR. LINGHORNE reported that Mr. O'Connell has now left the Banting Institute and gone to the Defence Research Board.

THE CHAIRMAN asked Dr. Macdonald to review Mr. O'Connell's report, and summarize the findings at the Committee's second session. This he agreed to do.

DR. MACDONALD later presented a very complete summary of Part II of the investigation.

DR. HAMILTON expressed surprise that no attempt had been made to study the exudate except from smear; he thought that there might well be other pathogenic organism which might be in part responsible for the infection.

DR. MACDONALD said they had been attempting to assess mixed infections. Suggestions in the literature had indicated the presence of several anaerobic organisms. In his laboratory they had been able to isolate up to 30 different organisms from one exudate. It was a long-term job and the initial problem needed to be solved before a study of mixed infections was undertaken.

Some discussion followed as to the relative merit of the investigation being carried on by Mr. O'Connell, and the view was expressed that a little more direction would be helpful.

11. DR 23 - Dr. C.P. Leblond

"Entry of phosphate and carbonate salts into teeth as shown with the help of radio-phosphorus and radio-carbon."

A summary and report of this work appear in APPENDIX "O"

12. DR 26 - Dr. R.E. Moyers

"A study of the normal and abnormal physiologic forces of the oral cavity."

THE CHAIRMAN stated that a letter had been received from Dr. Moyers on this project (which has been completed), giving some further information as to the use which has been made of the strain-gauge analyzer purchased under this grant. An excerpt of this letter appears in APPENDIX "L"

13. DR 27 - Dr. J.W. Abbiss and Dr. A.G. Nutlay

DR. BAGNALL said that while the work had been completed under this project, he understood that Dr. Nutlay plans to send a further report to the Committee. However, Dr. Nutlay has been ill and has been unable to complete the report for presentation.

14. DR 28 - Dr. O.J. Walker

"Development of a method for determining fluorine by means of a photo-electric colorimeter."

DR. WALKER presented the summary and complete report which appear in APPENDIX "K"

In the discussion which followed, mention was made of difficult translations of scientific articles carried out from time to time by grantees, and Dr. Thomson suggested that copies of such translations should be filed with the National Research Council for inclusion in a list of translations that is being maintained.

15. DR 33 - Dr. H.A. Hunter

"The investigation of the mechanism concerned in the deposition of lime salts formed in bridging over a pulp exposure."

DR. HUNTER presented the summary and report of his work which appear in APPENDIX "D"

THE CHAIRMAN said that this investigation was of great interest, and the Committee would hope that the work could be continued even though Dr. Hunter does not intend to make application for further funds from the Committee in the coming year.

16. DR 34 - Dr. J.P. Lussier

"Studies of calcium metabolism in teeth with the aid of Ca^{45}."

A summary and report on this project appear in APPENDIX "M"

THE SECRETARY informed the Committee that Dr. Lussier had written in January stating that he did not wish to renew his grant under DR 34 for the coming year, as Dr. D'Iorio is on indefinite leave at Madison, and he himself is leaving shortly for San Francisco for a two-year study period.

17. DR 35 - Dr. J.B. Macdonald

"Experimental fusospirochetal infection with mixtures of pure cultures."

A summary and report on this project appear in APPENDIX "G"

DR. MACDONALD stated that he is preparing two papers for publication and will write a third when certain other tests have been made. These will deal respectively with the following subjects:-

(i) method of morphologic identification; (ii) method of isolation of organisms; (iii) key to recognition of organisms reported in the literature over many years.

He also requested that the title of his project be changed to read as follows:- "Bacteriology of periodontal disease. - (1) Experimental fusospirochetal infection with mixtures of pure cultures."

It was AGREED that this change should be made on the application for renewal of the grant.

DR. HAMILTON suggested that the cause of death or organisms might be due to the formation of some toxic substance produced in the broth by the organisms.

18. DR 36 - Dr. G. Nikiforuk

"Demineralization of hard tissues as bone and teeth by organic chelating agents."

Dr. Nikiforuk presented his report on this project which appears in APPENDIX "C"

19. DR 37 - Dr. R.E. Moyers

"Further studies in the electromyography of the head, face and neck".

THE CHAIRMAN presented the summary and report which appear in APPENDIX "J"

He suggested that the Committee might ask Dr. Moyers to attend the September meeting and present a report on his project, if his grant is renewed.

(Note by the Secretary - On 5 March, 1952, a news item reported that Dr. Moyers had been presented with the Golden Cross of Phoenix "for services rendered to Greece during the enemy occupation and after." He parachuted into the mountains of the province of Evrytania, Greece, during the German occupation of World War II. He acted as liaison between the Resistance Movement in Greece and the Near East Allied Command. While there, he established the first Health Unit which the Resistance Movement lacked entirely.)

20. DR 38 - Dr. K.J. Paynter

"The effect of thiouracil on the development of molar teeth of rats."

DR. PAYNTER presented his own summary and report which appear in APPENDIX "E"

DR. THOMSON suggested that reference should be made to Dr. Hector Mortimer's techniques as published in "Radiology."

DR. BAGNALL said that he had recently had a most interesting case of a twelve-year old child (a hypothyroid case) which he hoped to write up shortly.

21. DR 39 - Dr. A.G. Racey

"Sectioning of teeth and surrounding structures."

THE CHAIRMAN presented Dr. Racey's summary which appears in APPENDIX "F"

He said that a casual examination of the report suggested that the work had been mostly directed to the improvement of techniques. He thought that if Dr. Racey's grant was renewed it might be useful to ask him to come to the next meeting to discuss his project.

22. DR 40 - Dr. L.A. Funt

"Effect of tooth eruption and function on the growth of the mandible and facial complex of cats."

THE SECRETARY said that no report had been received from Dr. Funt.

23. DR 41 - Dr. H. Jenkins

"A study of the dental arch form and certain of its anatomical relations in a series of clinically excellent dentitions."

A brief summary of the project appears in APPENDIX "A"

24. Fellowships

THE CHAIRMAN said that two fellowships had been awarded by the Committee this year and interim reports had been secured regarding the work done to date under these awards.

(i) Dr. Willa Liu Chou

THE CHAIRMAN stated that Dr. Moyers who is supervising Dr. Liu's work under the fellowship had written to say that there had been some difficulty in securing a United States visa for Dr. Liu, and as a consequence it had not been possible for her to visit Western Reserve University, where she had hoped to study a series of X-rays used at that institution in making cranial measurements. He said that Dr. Liu thought she should not ask for the remainder of her fellowship grant until such time as it would be possible for her to visit the United States.

Dr. Ellis had asked that this matter be brought to the attention of the Committee and had suggested that the remainder of the fellowship grant be made available this year to Dr. Liu, and that a letter be written to Dr. Moyers asking him to see that the best possible use was made of the money. It was understood that the Awards Office did not favour the transfer of fellowship funds to the next ensuing year.

It was MOVED by Dr. Macdonald and SECONDED by Dr. McDougall:

That the remainder of the fellowship grant payable to Dr. Liu be sent to the bursar at the University of Toronto, and that a letter be written to Dr. Moyers expressing the hope that the remaining funds under the fellowship can be used profitably either in the continuation of the present project or on some new work that he may suggest.

CARRIED.

(ii) Dr. Gerald Shklar

THE CHAIRMAN stated that a letter had been received 30 January, 1952, from Dr. Irving Glickman regarding the work being done by Dr. Gerald Shklar under his fellowship. The letter from Dr. Glickman appears in APPENDIX "P"

It was gratifying to know that Dr. Shklar was making good progress under his fellowship award.

25. Finances

THE CHAIRMAN asked for a statement of the Committee's finances.

THE SECRETARY reported that as at 29 February, 1952, the financial standing of the Committee was as follows:-

Excluding $5,000 set aside for fellowships, the amount awarded to the Committee by NRC for 1951-52 was		$35,700.00
Of this sum, a total of ..... was in the possession of the grantees 1 April, 1951, and was therefore a first charge against their awards for 1951-52, leaving a balance of		2,846.83 $32,853.17
However, there was revoted of the sum in the hands of grantees, a total of		1,965.43
So that the total available to the Committee was		$34,818.60
Expenditures of.....	$24,823.23	
and commitments of....	8,517.31	
to 29 February, 1952, add up to	$33,340.54	33,340.54
Leaving a free balance of		$ 1,478.06

26. Letter from Canadian Dental Association (Publication of papers)

THE CHAIRMAN said that a letter had been received from the Canadian Dental Association 24 January, 1952, requesting consideration of the publication of research reports relating to work sponsored by the Committee. Dr. Gullett's letter pointed out that the CDA Committee on Publications felt that Canadian literature was losing out through the publication of dental research papers in journals outside Canada. It was fully understood that it was often desirable to have a report published in a journal in the United States or elsewhere, but it was also thought that such reports should appear concurrently in the Journal of the C.D.A.

DR. GARVIN said that it was desired to stimulate interest in the Journal of the Canadian Dental Association. He suggested also that

American journals dealing with dental research do not have a wide circulation in Canada, and work published in them, therefore, frequently does not come to the attention of Canadians.

THE CHAIRMAN suggested that most journal editors are unwilling to accept papers for publication that are to appear in other journals concurrently.

DR. HAM doubted that many Canadian dentists would be interested in reading the pure research articles that are ordinarily submitted to outside journals. He thought perhaps a solution might be found in having a well informed person write a review of a current research project in semi-popular form for publication in the Journal of the Canadian Dental Association.

DR. RAE and DR. MACDONALD both pointed out that they publish their papers in the journals which are used by other workers in the same fields in order that they may obtain the help of such contemporary workers.

THE CHAIRMAN said that the suggestion for the publication of review articles seemed to be quite acceptable and he suggested that a reply be sent to Dr. Gullett pointing out that the work arising from grants-in-aid awarded by the Committee was frequently not suitable for publication in the Journal of the Canadian Dental Association, and for this reason it was customary to submit some of the Committee's papers to other journals. He thought that the editor might be informed of the proposal to prepare review articles which would be more appropriate for publication in his journal. (Later the Chairman drafted a letter and read it to the Committee, who approved of it being forwarded to Dr. Gullett.)

27. Air Travel, 1951-52 (Min. 24, 14th Mtg.)

It was AGREED to include the names of Dr. O.J. Walker and Dr. W.J. Linghorne among those for whom air travel is authorized for 1951-52.

28. Air Travel, 1952-53

It was MOVED by Dr. Hamilton and SECONDED by Dr. Macdonald:

> That air travel in 1952-53 be authorized for Dr. J.S. Bagnall, Dr. A.C. Burton, Dr. M.H. Garvin, Dr. H.R. Maclean and Dr. R.A. McDougall, and that the Executive be empowered to deal with any further cases of air travel as they may arise during 1952-53.
>
> CARRIED.

The first session was adjourned at 5 p.m.

SECOND SESSION

9:30 a.m., Tuesday, 4 March, 1952.

29. Attendance

Dr. J. Stanley Bagnall
Dr. A.C. Burton
Dr. M.H. Garvin
Dr. Arthur W. Ham
Dr. J.D. Hamilton
Dr. L.A. Kilburn
Dr. J.B. Macdonald
Dr. R.A. McDougall
Dr. J.J. Rae
Dr. D.L. Thomson

Mr. S.J. Cook, Secretary

By invitation

Dr. H.A. Hunter
Dr. Gordon Nikiforuk
Dr. K.J. Paynter
Dr. O.J. Walker

30. Membership of the Committee (Min. 34, 12th Mtg.)

THE CHAIRMAN reported that four members of the Committee will have completed their present terms of appointment 31 March, 1952. Of these Dr. Bagnall has served only a two-year term; Dr. McDougall and Dr. Ham have both served one partial term and one full term of three years. These three are, therefore, eligible for nomination for a further term of three years. Dr. Collip has served one partial term and two full terms and is, therefore, under the Committee by-laws ineligible for re-appointment until at least one year has elapsed.

It was MOVED by Dr. Hamilton and SECONDED by Dr. Garvin:

> That Dr. Bagnall, Dr. Ham and Dr. McDougall be nominated to the National Research Council for another three-year term of membership to 31 March, 1955.

CARRIED.

After some discussion it was MOVED by Dr. Rae and SECONDED by Dr. Ham:

> That the nomination of a member to succeed Dr. Collip on the Committee be left to the Executive with the suggestion that consideration be given in the selection of a candidate to one who has had training in pharmacology or nutritional biochemistry, and that if possible a candidate be chosen who would also represent Queen's or a French-Canadian university. CARRIED.

THE CHAIRMAN agreed to consult the members of the Executive and to take responsibility for this item.

Subsequently, Dr. D.L. Thomson conferred with Dean Charron and submitted recommendations to Dr. Roy G. Ellis, who in turn advised the Secretary that it had been agreed to nominate Dr. Edouard Page, Director, Department of Nutrition, Medical School, Laval University, Quebec, Que.

THE SECRETARY ascertained that Dr. Page was willing to serve in this capacity and accordingly submitted his name to Council.

THE CHAIRMAN paid tribute to the assistance which had been given to the Committee throughout his term of office by Dr. Collip. His advice on biochemical and physiological subjects had always been very helpful, and his long experience in other committees, and as a former member of the National Research Council, had enabled him to make a substantial contribution to the Committee's deliberations. Unfortunately, under the regulations it was not possible to re-nominate him for a further term of membership at this time.

31. Applications for Grants, 1952-53 (Min. 22, 14th Mtg.)

THE CHAIRMAN pointed out that last year the Committee had made use of some of its residual funds for the purchase of equipment requested by grantees that could be bought before the end of the fiscal year. Some thought was given to the adoption of a similar practice at this time but it was decided that the difficulties involved were too great to make it worth while. Applications were then considered and dealt with as follows:-

RENEWALS

Grant No.	Grantee	Amount $
DR 1	Dr. J.J. Rae ($1080) Subsequently, by Letter Ballot of the Executive, this grant was increased by $450 to provide for three months summer employment of an extra technician. The total grant therefore was	1530
DR 13	Dr. M. Doreen Smith The Committee AGREED to approve the amount asked for under the pablum problem, and to make an	500

Grant No.	Grantee	Amount

additional grant later if it appears useful to pursue this project further. The Executive was empowered to consult with Dr. Brown as to the desirability of carrying on tooth studies under a Committee grant after June, 1952.

DR 22 — Dr. C.H. Best — 4,200

The Chairman read a letter from Dr. Best to Dean Ellis dated 12 February, 1952, in support of this application, and stating that if continued support from the Committee could be ensured for this project for the next four or five years it would be a thoughtful and encouraging action "that would be productive of the very best that Linghorne has to give."

DR. RAE pointed out that the Committee's funds are provided on an annual basis which precludes a making of commitments over a period of years.

DR. MACDONALD felt that the work was well planned and well organized.

DR. HAM expressed the opinion that this project represented good sound basic work, but that if Dr. Linghorne is to do it, he needs a full-time technician to cut sections.

THE CHAIRMAN reminded the Committee of the discussion regarding this grant a year ago, and suggested that if the Committee felt that Dr. Linghorne's presentation warranted the continued support of this project the grant might be made for this year without committing us in the future years, but with an expression of encouragement.

On motion of Dr. Ham seconded by Dr. Hamilton it was AGREED to make the grant and to send a note of interest

Grant No.	Grantee	Amount

Grant No.	Grantee	Amount
	and sympathy in the project with the hope that it would be continued to a successful conclusion.	
DR 23	Dr. C.P. Leblond	3,000
DR 28	Dr. O.J. Walker	600
DR 35	Dr. J.B. Macdonald	3,300
	At Dr. Macdonald's request the title of his project was changed to read as follows: "Bacteriology of periodontal disease. - I. Experimental fusospirochetal infection with mixtures of pure cultures."	
DR 36	Dr. G. Nikiforuk	1,600
DR 37	Dr. R.E. Moyers	800
	THE CHAIRMAN suggested that Dr. Moyers be invited to attend the next meeting of the Committee.	
DR 38	Dr. K.J. Paynter	2,610
DR 39	Dr. A.G. Racey	
	An application for renewal of the grant in the amount of $1,600 was deferred pending further inquiry to be made by the Executive.	
	(Later approved by Letter Ballot)	1,600
	Total Renewals	$19,740

New applications

Grant No.	Grantee	Short Title	Amount
DR 42	Dr. Gordon Nikiforuk, Assoc. Prof. of Pedodontics, Dept. of Dental Research, University of Toronto, Toronto, Ont.	Studies in the chemistry of the saliva. - II. The reduction of methylene blue by saliva.	$1,500

DR. NIKIFORUK explained that the organisms within the saliva seemed to be the active agent. Basic studies should be made first.

Grant No.	Grantee	Amount
	and sympathy in the project with the hope that it would be continued to a successful conclusion.	
DR 25	Dr. C.P. Ledoux	3,000
DR 26	Dr. O.J. Walker	600
DR 35	Dr. J.B. Macdonald	3,300
	At Dr. Macdonald's request the title of his project was changed to read as follows: "Bacteriology of periodontal diseases. - I: Experimental fusospirochetal infection with mixtures of pure cultures."	
DR 36	Dr. O. Nikiforuk	1,600
DR 37	Dr. R.E. Moyers	800
	THE CHAIRMAN suggested that Dr. Moyers be invited to attend the next meeting of the Committee.	
DR 38	Dr. E.J. Payster	2,010
DR 39	Dr. A.O. Racey	
	An application for renewal of the grant in the amount of $1,800 was deferred pending further inquiry to be made by the Executive.	
	(Later approved by Dexter Bellot)	1,800
	Total Renewals	$19,710

New applications

Grant No.	Grantee	Short Title	Amount
DR 42	Dr. Gordon NIKIFORUK, Assoc. Prof. of Pedodontics, Dept. of Dental Research, University of Toronto, Toronto, Ont.	Studies in the chemistry of the saliva. - II. The reduction of methylene blue by saliva.	$1,50

DR. NIKIFORUK explained that the organisms within the saliva seemed to be the active agent. Basic studies should be made first.

He knows that older people reduce it faster than children but he does not know what the organisms are.

Doctors RAE and BURTON both suggested that the grantee consult Dr. Wynne, of the University of Toronto, who is knowledgeable on this subject.

THE CHAIRMAN suggested that Dr. Stavraky, professor of physiology, at the University of Western Ontario, might also be consulted.

The applicant had asked for $50 for travel expenses, but he was informed that such expenses can be met out of the Committee's travel grant on application to the Chairman. The application had, therefore, been reduced by this amount.

DR 43	Dr. S.G. Davis, Assoc. Prof. of Chemistry, University of Alberta, Edmonton, Alta. and Dr. H.R. MacLean, Prof. Operative Dentistry, University of Alberta, Edmonton, Alta.	Electro potentials between dental alloys	$150
DR -	Dr. R.E. Moyers, Prof. and Head, Department of Orthodontics, Faculty of Dentistry, University of Toronto, Toronto, Ont.	Experimental Ankylosis of teeth.	$825

After some discussion on this application it was MOVED by Dr. Ham and SECONDED by Dr. Rae:

> That a decision on this project be deferred pending inquiry by Dr. R.G. Ellis, and consideration by the Executive.

(Dr. Ellis later reported that Dr. Moyers wished to withdraw this application, in order that he might further document the project.)

Summary

Renewals (10).....	$19,740
New applications (2)	1,650
Total	$21,390

32. Fellowship Applications

THE CHAIRMAN expressed regret that with the exception of a request from a practising dentist in San José, Costa Rica, named Dr. Erich Keibel, there were no applications in hand for the dental fellowships offered by the Committee.

The letter and enclosures submitted by Dr. Keibel were considered.

THE SECRETARY was directed to advise Dr. Keibel that applicants for graduate dental research fellowships must be graduates from a dental school approved by Council and resident in Canada.

33. New Projects

DR. GARVIN suggested that the effect of light upon foodstuffs in relation to dentistry be added to the Committee's list of new projects. He said that he had observed the effects of cod-liver oil on panchromatic films, and as a result he had been led to think of the possible effect of sunlight on foods exposed in store windows; he considered this would be an interesting field of study from the dental standpoint. He pointed out that the vitamin content of butter from a certain Regina source was said to be higher than in butter from other places.

DR. PAYNTER mentioned that work is being done on the effect of vitamins in relation to teeth and especially concerning the incidence of caries at Toronto.

34. Date of next meeting

It was AGREED that the next meeting of the Committee should be held on Monday, 29 September, 1952.

The meeting adjourned at 12:30 p.m.

APPENDIX "A"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: A study of the dental arch form and certain of its anatomical relations in a series of clinically excellent dentitions.

Grantee: Dr. H. Jenkins

Project No. DR 41 Amount of Grant: $300

SUMMARY OF WORK

To date I have examined approximately 2,000 university students and selected 35 with clinically excellent dentitions. However the gathering of records of these students has been seriously hindered by University examinations and the Toronto street car strike.

In view of the impossibility of carrying out this programme this year, I am not drawing on this grant this year. A fresh application for this money will be made when the occasion for the completion of this research programme arises.

January 21, 1952.

APPENDIX "B"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: Investigation of materials for use as cements for methacrylate inlays (DR 12); investigation of methods for the use of methyl methacrylate as direct dental fillings (DR 25).

Grantee: Dr. A.E.R. Westman

Project Nos.: DR 12 and 25 Amount of Grant: $4,000

These investigations were terminated as of 30 June, 1951, and a report made to the Committee in September, 1951, will therefore serve as the final report on the projects.

Since the last meeting another paper has been added to the series arising from these projects. This one is entitled "Acrylic Fillings - General Comments and Some Experimental Data", by D.R. Christie.

21 January, 1952.

APPENDIX "B"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: Investigation of materials for use as cements for methacrylate inlays (DH 12); investigation of methods for the use of methyl methacrylate as direct dental fillings (DR 25).

Grantee: Dr. A.E.R. Westman

Project Nos.: DR 12 and 25 Amount of Grant: $4,000

These investigations were terminated as of 30 June, 1951, and a report made to the Committee in September, 1951, will therefore serve as the final report on the projects.

Since the last meeting another paper has been added to the series arising from these projects. This one is entitled "Acrylic Fillings - General Comments and Some Experimental Data", by D.R. Christie.

21 January, 1952.

APPENDIX "C"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: Demineralization of hard tissues by organic chelating agents.

Grantee: Dr. G. Nikiforuk

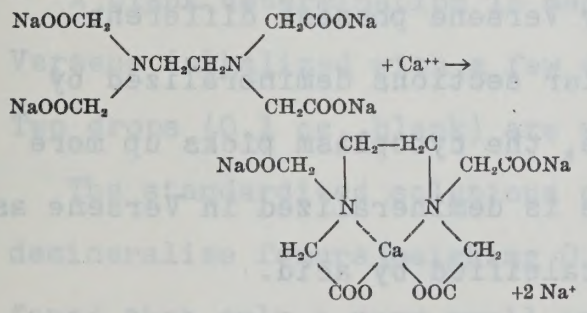
Project No. DR 36 Amount of Grant: $2400.
350. (additional)

PRELIMINARY REPORT

A preliminary statement regarding the Demineralization of Hard Tissues by Organic Chelating Agents appeared in Science, 113:560, 1951, of which a reprint appears below.

Demineralization of Hard Tissues by Organic Chelating Agents

THE sodium salts of ethylene-diamine tetracetic acid are noncolloidal organic chelating agents that resemble the inorganic polyphosphates (e.g., sodium hexametaphosphate) in their ability to form soluble nonionic chelates with a large number of metallic ions. The reaction with calcium is thought to proceed as follows:



The resulting complex is stable over a wide range of temperature (*1*). Complex compounds with calcium and other heavy metal ions are formed at variable hydrogen ion concentrations. These compounds are formed most efficiently in alkaline solutions (*2*). Since decalcification of bone and other hard tissues has hitherto only been accomplished in acid media, it was of interest to see whether these chelating agents would make a suitable demineralizing medium.[1]

Preliminary investigations have shown that demineralization in saturated aqueous solutions at pHs ranging from 5.0 to 10.3 can be accomplished. The solubility ranges from 11 g/100 ml water for the disodium salt (pH 5.0) to 103 g/100 ml water for the tetrasodium salt (pH 10.3). The time required is about the same as that needed for a comparable specimen in the formic-citric acid method of Morse (*3*), and slightly longer than that required for 5% HNO_3. Preservation of structural detail and of the staining properties of the tissues seems excellent. This was particularly evident in the elements of the pulp and bone marrow. Further experiments to determine the optimal pH and concentration of the chelating compounds for the preservation of enzymes and tissue elements are now being conducted.

LEO M. SREEBNY

Division of Oral Pathology
College of Dentistry
University of Illinois, Chicago

GORDON NIKIFORUK

Department of Pedodontia
Faculty of Dentistry
University of Toronto

References

1. SCHWARZENBACH, G., and ACKERMAN, H. *Helv. Chim. Acta*, **30**, 1798 (1947).
2. *Powerful Organic Chelating Agents.* Tech. Bull. No. 2. Framingham, Mass.: Bersworth Chemical Company.
3. MORSE, A. *J. Dental Research*, **24**, 143 (1945).

[1] The chelating agents used in this work were obtained from Alrose Chemical Company, Providence, R. I., and the Bersworth Chemical Company, Framingham, Mass.

EXPERIMENTAL FINDINGS

A. Effect of pH on Complexing Power of Versene

(refer to previous report)

B. Demineralization of Hard Tissues by Versene: Preliminary Experiment

(refer to previous report)

C. Effect of Concentration on Rate of Bone Demineralization by Versene

(refer to previous report)

D. Effect of pH on Rate of Bone Demineralized by Versene

(refer to previous report)

E. Histological Findings

Only a few sections are available. These suggest the following:

a. Preservation of staining properties are excellent, particularly in the bone marrow.

b. The specimens demineralized by Versene possess different staining properties than similar sections demineralized by 5 per cent HNO_3. For example, the cytoplasm picks up more haematoxylin stain when tissue is demineralized in Versene as compared to similar tissue decalcified by acid.

c. Tissue demineralized by Versene is "harder". This may be due to removal of less organic material from bone than occurs when a specimen is decalcified by acid. Thus teeth are sectioned with great difficulty when embedded in paraffin.

The paraffin apparently does not penetrate into the tooth tissue, permitting the tissue to dry and harden while in the oven. The histological phase of this work awaits further investigation.

F. Effect of Citrate on Rate of Demineralization of Versene

No appreciable difference in the rate of demineralization was observed by neutralizing the sodium salt of ethylene diamine tetra-acetate with citric acid to a pH of 7.

G. Amount of Versene Chelated During Demineralization of Bone Specimens

A solution of Versene was standardized by the following procedure:

To a 10 cc. sample of Versene, as supplied by Bersworth Chemical Company, diluted to 100 cc. with distilled water, is added 10 cc. of 5 per cent ammonium oxalate indicator and the resulting solution is titrated until the first detectable permanent turbidity is observed.

A blank determination is made with distilled water in place of Versene (alkalized with a few drops of 10 per cent NaOH solution). Two drops (0.1 cc. blank) are usually necessary to produce turbidity.

The standardized solutions of Versene (500 cc.) were used to demineralize femurs (weighing 0.330 g.) of guinea pigs. It was found that only a very small amount of Versene was used during demineralization. It was decided that for comparable specimens there is no need to change the solution during demineralization.

H. Rate of Demineralization and Height of Versene Column

The calcium complex of Versene has a higher density than the original solution. Experiments were designed to test the increasing demineralization when bone specimens are suspended near the surface of a high column of reagent as compared to dropping it to the bottom of the vessel. Definite increases in the speed of demineralization were noted if the material is suspended near the upper surface of the high columns.

APPENDIX "D"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1952

Subject: The investigation of the mechanism concerned in the deposition of lime salts formed in bridging over a pulp exposure.

Grantee: Dr. H. A. Hunter

Project No.: DR 33 Amount of Grant: $1600

SUMMARY OF WORK

This report contains a histological study and analysis of sixty-eight dogs' teeth that were experimentally treated with pulp capping materials of seven kinds: $Ca(OH)_2$; $Mg(OH)_2$; "Jodoformagen"; calcium oleate; cholesterol; zinc oxide-eugenol; and "Denco" temporary cement.

All the treated teeth in the groups using calcium oleate, cholesterol, and "Denco" temporary cement, failed to show any stimulation of active dentine deposition. One tooth out of ten treated with "Jodoformagen" showed a slight amount of calcific bridging. The two groups using calcium and magnesium hydroxide showed a lot of active deposition, and those cases where none was produced, could be accounted for by the intervention of pulp necrosis or suppuration. The high pH common to both these groups is an obvious lead to pursue using other materials having the same tendency to elevate the pH of the tissue fluids.

February 7, 1952.

APPENDIX "D"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: The investigation of the mechanism concerned in the deposition of lime salts formed in bridging over a pulp exposure.

Grantee: Dr. H. A. Hunter

Project No.: DR 33

I have not made application for further funds for the coming year. For the past few months, my laboratory functions have been at a virtual standstill, due to alterations in our building. The supplies on hand, I hope, will be adequate, and my technician is currently being supported by University funds.

29 Jan. 1952.

29 Jan. 1952.

APPENDIX "D"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1952

Subject: The investigation of the mechanism concerned in the deposition of lime salts formed in bridging over a pulp exposure.

Grantee: H. A. Hunter

Project No.: DR 33 Amount of Grant: $1600

ANNUAL REPORT

The project in this investigation is to develop an approach towards an understanding of the underlying mechanism whereby lime salts are deposited in the bridging-over of pulp exposures. To this end, varying agents were placed against exposed pulps to observe their effects on this mechanism.

The test materials used were: calcium hydroxide; magnesium hydroxide; "Jodoformagen"; calcium oleate; zinc oxide-eugenol cement; cholesterol; and "Dencо" cement. These were applied over intervals ranging from one to nine weeks.

A survey of the histological findings of these treated teeth (see previous schedule, Appendix F-3) showed no visible evidence that could be interpreted as having been induced by calcium oleate in any of the fifteen treated teeth. The same could be said for the teeth treated with cholesterol, nor did any of the four cases of "Dencо" cement show any deposition about the exposure. In the case of zinc oxide-eugenol treated teeth, the two remaining for nine weeks both showed calcific bridging over the exposure and about dentine splinters embedded in the pulp tissue. Of the ten teeth treated with "Jodoformagen", only one showed a trace of deposition; one pulp was necrotic; and four showed suppuration and no evidence of bridging; and the remaining four showed no signs of active calcific deposition.

The two remaining test groups using calcium and magnesium hydroxide showed active deposition of calcific material. In those cases where it did not occur, it was explainable by the presence of suppuration. In one case lasting nine weeks, there was evidence of resorption of the margin of the dentine border, but this same resorption could not be seen in the other cases using calcium hydroxide. There was no evidence of resorption of the primary dentine prior to deposition of the secondary dentine. It was noticed that the greatest thickness of deposition was about the periphery of the exposure.

There are two forms of the material deposited in the pulp: 1) A homogeneous, largely atubular calcified matrix seen about dentine splinters in the traumatized areas.

2) Histologically recognizable tubular dentine complete with adjoining layer of odontoblasts. The former may be merely an atubular form of dentine, not necessarily differing in its mode of deposition.

Discussion

The extent of deposition activity was almost the same in the calcium and magnesium hydroxide groups, the calcium group being slightly more active. This, then, cannot be due to the addition of calcium ions alone. The obvious common factor is their pH, that of $Ca(OH)_2$ being about 12.0 and that of $Mg(OH)_2$ about 10.3. Both of these are well above the pH 7.4 of tissue fluids. The slight added activity of $Ca(OH)_2$ might be attributed to its higher pH, though proof is lacking at the moment.

The three groups of materials; viz., calcium oleate; cholesterol; and zinc oxide-eugenol, which for the most part showed no active deposition of calcific material, have as one of their common factors no pH since they are not in aqueous solution.

Linghorne[1] (1951) noticed that, in areas where resorption of bone grafts was taking place, this resorption seemed to stimulate the primitive mesenchymal cells to differentiate into osteoblasts. One possible explanation for this is that these same undifferentiated mesenchymal cells were in a substrate having a high calcium ion content due to this resorption. In the teeth treated for nine weeks with zinc oxide-eugenol, the resultant bridging and dentine deposition has been explained by Manley[2] (1946) where he calls these dentine splinters "organizers" responsible for the differentiation of the primitive mesenchymal cells into osteogenic functioning cells. On this basis, in the pulp where we have these same undifferentiated mesenchymal cells in a relatively primitive tissue, the calcium ion content of the tissue fluid could be increased by the pH of the fluid in the pulp. Therefore, the two materials, calcium and magnesium hydroxide, were used for this purpose, which might be expected to have the effect of increasing the calcium ion content. The magnesium hydroxide was chosen for its similar pH effect without adding calcium ions.

The results seem to indicate that both materials favoured deposition of a calcified material and stimulated throughout the pulp an increased activity of the odontoblasts. The obvious lead to follow is to try other materials that tend to elevate the pH of tissue fluids and see if we get a relationship between elevated pH and dentine deposition activity.

From the results of this experiment and that of others, the role of infection is important. So much so, that any form resulting in suppuration is a contra-indication of successful treatment: the pulp tissue dies before any rebuilding of the dentine can take place. In this regard, calcium hydroxide is believed by some workers to possess antibacterial properties.

Hermann[3](1930), who introduced calcium hydroxide as a capping material under the proprietary name of Calxyl, claimed that it possessed bacteriocidal action in dilution of 0.02% and that this is not due to its alkaline action since equivalent alkalinity of other substances are not bacteriocidal. He claims it to be one thousand times more disinfectant than tricresol-formalin. This was put to the test by incubating examples of calcium and magnesium hydroxide each on blood agar plates inoculated with staphylococcus aureus in the one case and streptococcus viridans in the other. None of the four plates showed any bacteriostatic action and this concurs with Reich's contention.

Conclusion

In this group of sixty-eight experimentally treated dental pulp exposures using different medicaments, the four groups using calcium oleate, cholesterol, "Denco" cement, and zinc oxide-eugenol cement, accounted for thirty-five teeth, two of which treated with zinc oxide-eugenol for nine weeks, were the only ones showing calcific activity about the exposure. One out of ten teeth treated with "Jodoformagen" showed active healing. In the calcium hydroxide group of thirteen teeth, five showed good deposition activity, and the remainder failed either because of intervening suppuration or necrosis of the pulp. In the magnesium hydroxide group, there were five active cases of deposition of dentine, one bifurcation involvement with suppuration, one pulp torn out in preparation, one necrotic pulp, and two cases of suppuration.

References.

1 Linghorne, W.J. - Personal communication.

2 Mainley, E.B. - Dental Structure and Dental Caries. Proc.Roy. Soc.Med. Sect. Odont. 39: 637-640 Aug. 1946

3 Hermann, B. W. - Dentin Obliteration der Wurzerlkanale nach Behandlung mit Kalzium. Z. Rundschau 39: 890-899, 1930.

4 Reich, J. - Basic Considerations Underlying the Treatment of Exposed Vital Pulps in the Teeth of Children. Aust. Jour. Dent. 55: 171-175 June 1951.

Acknowledgments The Grantee wishes to express his appreciation to the Banting and Best Department of Medical Research for the generous use of their facilities during the conduct of this work and to Mr. D.C. O'Connell for his part in expediting the laboratory work. Especial thanks are extended to Dr. W.J. Linghorne of the above Department for his charity in planning the project and assessing the results.

APPENDIX "E"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: The effect of thiouracil on the development of molar teeth of rats.

Grantee: Dr. K.J. Paynter

Project No.: DR 38 Amount of Grant: $1850

SUMMARY OF WORK

Some of the effects of the anti-thyroid drug, Thiouracil, on the developing molar teeth of the rat, have been studied. The teeth and jaws of hypothyroid rats of various ages from birth to twenty-five days have been examined both histologically and by means of the Grenz ray, and compared with normal, littermate, control animals of the same ages.

The reduction in over-all size, the delay in eruption, and the retardation in tooth formation associated with hypothyroidism has been confirmed.

The increase in density of the cancellous bone of the mandible reported by Becks, Collins, et al in 1948 in older animals, has also been observed here.

There has been observed also, a noticeable reduction in the number of osteoclasts around the roots of the developing molar teeth.

February 5, 1952.

APPENDIX "E"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: The effect of thiouracil on the development of molar teeth of rats.

Grantee: Dr. K.J. Paynter

Project No.: DR 38 Amount of Grant: $1850

REPORT OF WORK

Introduction:

This work represents the first of a series of investigations which are to be grouped under the general heading - "The Effects of the Endocrines on the Teeth and Jaws".

In this experiment some of the effects of a thyroid hormone deficiency have been observed.

Method of Procedure:

Newborn rats were rendered hypothyroid by subcutaneous administration of 6-n-propyl-2-thiouracil dissolved in 0.9% saline. Control, litter-mate animals, received an equal amount of 0.9% saline subcutaneously.

The rats were sacrificed in pairs, (control and experimental) when five, ten, fifteen, twenty and twenty-five days old. Two pairs of animals were killed on each of these dates and their heads, and pituitary and thyroid glands were prepared for study.

One-half the mandible of each animal was dissected out, and a grenz-ray picture was taken of each.

The remainder of the head was split sagittally and the complete half head decalcified and paraffin embedded for histologic section sagittally. The remaining maxilla was decalcified and prepared for section coronally. All sections were made serially and stained with hematoxylin and erythrosin.

Observations:

1. Evidence of thyroid hormone insufficiency was determined from the daily weight record of the animals, and from histologic sections of the pituitary and thyroid glands.

APPENDIX "B"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: The effect of thiouracil on the development of molar teeth of rats.

Grantee: Dr. K.G. Paynter

Project No.: DR 36 Amount of Grant: $1850

REPORT OF WORK

Introduction:

This work represents the first of a series of investigations which are to be grouped under the general heading - "The Effects of the Endocrines on the Teeth and Jaws".

In this experiment some of the effects of a thyroid hormone deficiency have been observed.

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One-half the mandible of each animal was dissected out, and a trans-ray picture was taken of each.

The remainder of the head was split sagittally and the complete half head decalcified and paraffin embedded for histologic section sagittally. The remaining maxilla was decalcified and prepared for section coronally. All sections were made serially and stained with hematoxylin and erythrosin.

Observations:

1. Evidence of thyroid hormone insufficiency was determined from the daily weight record of the animals, and from histologic sections of the pituitary and thyroid glands.

2. Grenz-Ray Studies::

a) Evidence of retardation in calcification of the molar teeth is evident in the grenz-ray as early as five days. This is four days after thiouracil injections were started.

b) After ten days of age, retardation in over-all size of the mandible becomes apparent. This discrepancy in size between control and experimental increases with age.

c) By fifteen days, the distal end of the incisor tooth crypt of the experimental group is more anterior in relation to the second molar crypt than it is in the control. This difference also increases with age.

d) After ten days the cancellous bone of the experimental mandibles appears more dense than in the controls. As with other differences, this increases with age, and is most marked at twenty-five days.

3. Histologic Study:

To date, one pair of animals, (control and experimental) have been studied at each of five, ten, fifteen and twenty days. The remainder of the specimens are still in the process of being sectioned and stained.

a) Evidence of retardation of tooth development is apparent at five days. Slowing of eruption time can be observed later - at about fifteen days.

b) In enamel, retardation may be seen both in the deposition of the matrix, and in maturation.

c) The difference in density of the cancellous bone is apparent histologically at twenty days.

d) A reduction in the number of Osteoclasts is noticeable in the experimental animals in the region of the developing roots of the molar teeth.

This observation would suggest the possibility that the increase in density of the mandibular bone is a result of a reduction in rate of resorption.

It may be, however, that reduction in the number of osteoclasts may simply be another indication of the slower development of the molar roots in the hypothyroid animals.

February 5, 1952.

2. Cross-Ray Studies:

a) Evidence of retardation in calcification of the molar teeth is evident in the cross-ray on early as five days. This is four days after subcutaneous injections were started.

b) After ten days of are, retardation in over-all size of the mandible becomes apparent. This discrepancy in size between control and experimental increases with age.

c) By fifteen days, the distal end of the incisor tooth crypt of the experimental group is more anterior in relation to the second molar crypt than it is in the control. This difference also increases with age.

d) After ten days the cancellous bone of the experimental mandibles appears more dense than in the controls. As with other differences, this increases with age, and is most marked at twenty-five days.

3. Histologic Study:

To date, one pair of animals, (control and experimental) have been studied at each of five, ten, fifteen and twenty days. The remainder of the specimens are still in the process of being sectioned and stained.

a) Evidence of retardation of tooth development is apparent at five days. Slowing of eruption time can be observed later - at about fifteen days.

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d) A reduction in the number of Osteoclasts is noticeable in the experimental animals in the region of the developing roots of the molar teeth.

This observation would suggest the possibility that the increase in density of the mandibular bone is a result of a reduction in rate of resorption.

It may be, however, that reduction in the number of osteoclasts may simply be another indication of the slower development of the molar roots in the hypothyroid animals.

February 5, 1952.

APPENDIX "F"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952.

Subject: Sectioning of teeth and surrounding structures and the effects of potassium deficiency and of pyridoxine deficiency on the teeth and surrounding structures of dogs.

Grantee: Dr. A.G. Racey.

Project No.: DR 39 Amount of Grant: $1,600

SUMMARY OF WORK

The work carried out so far this year has been concentrated mainly on perfecting the decalcification and sectioning of dogs' teeth and surrounding structures; and, also, the effects of a potassium deficiency upon these tissues. Relatively little work was accomplished on the pyridoxine deficiency due to the difficulties encountered during sectioning and decalcification of normal dogs teeth and mandible and those of the potassium deficient dog. But this is now being carried on concurrently with the potassium deficiency.

1) Preparation of sections of dogs' teeth and surrounding structures.

Dental histological technique is seen to follow a definite pattern. Fixation, decalcification, washing, dehydration, clearing and finally infiltration and embedding in paraffin wax.

1. Decalcification. An ionic decalcifier was used which decalcifies the teeth in approximately 36 hours but which would not completely decalcify sections of the mandible in one week. They were never left in the decalcifier longer than 6 days because if they were, the soft tissue disintegrated.

2. Dehydration. After dehydration the tissue became harder and very difficult, if not impossible, to slice. This was improved slightly by shortening the dehydration time and also by soaking in "mollifax" overnight. But this tended to distort the tissue considerably.

3. Sectioning. Sections as thin as 6mu were obtained after the problem of decalcification and dehydration were eliminated, but there was too much distortion and so sections of 8mu were used. It was noted that the potassium deficiency was easiest to section than pyridoxine then normal.

4. Staining. Sections tended to become loose during staining but this was finally eliminated by dipping the slides in a 1/4% solution of celloidin after the removal of the paraffin. It was also noted that

sections of the pulp were difficult to obtain and this was thought to be due to a lack of penetration of the fixative. The tooth is now being drilled with a small burr in order to allow the penetration of the fixative into the pulp chamber.

The stains used are haematoxylin and eosin, phloxine, saffron and Van Gieson's stain for connective tissue.

2) Effects of potassium deficiency.

The findings so far seem to indicate that there is a lack of definition of the fibres of the periodontal membrane nor are they as well orientated as those of the normal dog. They appear to be more dense, yet a lesser number of bundles.

There also appears to be a tendency for the alveolar bone to become osteoporotic. The bony tissue appears to exhibit a regression in that there are large vacuolated areas present which contain a reticular network. Yet, the alveolar bone which is remaining appears to be denser with a more regular periosteum and less microscopic evidence of attachment of the periodontal fibres. The remaining alveolar bone and tooth substance appears to have the same affinity for staining.

3) Effects of pyridoxine deficiency.

There is nothing to report as yet on this aspect of the project though several sections have been obtained. But it is felt that several more should be obtained before any findings are reported. The reason for the lack of sections is the time consumed with the problems of decalcification and subsequent steps as previously mentioned.

This aspect will be fully covered in the final report. There will also be included some photomicrographs to show the difference between the normal and abnormal.

APPENDIX "G"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1952

Subject: Experimental fusospirochetal infection with mixtures of pure cultures

Grantee: Dr. J. B. Macdonald

Project No.: DR 35 Amount of Grant: $2800

SUMMARY

Cultural requirements, biochemical activity and pathogenicity of the motile anaerobes isolated from the oral cavity have been studied. All three Groups grow best at 37°C. but Group III grew over a wider range of temperatures (20° to 45°C.) than did Groups I and II (27° to 40°C.). Growth of Group II was enhanced in the presence of small amounts of carbon dioxide. Groups I and III seemed unaffected by this gas. Oxygen tolerance was tested by growth in shake tubes. Group I failed to grow in shake tubes, Group III grew throughout the greater length of the tubes and Group II showed a distinct preference for a zone slightly below the surface. Group II, thus appears to be microaerophilic though it will not survive successive subcultures in fluid media incubated aerobically. All three Groups grew as well or better at pH 7.2 than at other hydrogen-ion concentrations tested. Group III grew over the widest range of hydrogen-ion concentrations-from pH 4.5 to pH 8.6.

Biochemical tests included tests for fermentative capacity against three different sugars. Production of indol, production of hydrogen sulphide and reduction of nitrates to nitrites. Group I did not ferment any of the sugars tested. Groups II and III fermented dextrose and sucrose but not mannite. Indol was not produced from any of the three Groups. Hydrogen sulphide was produced by Group I but not by Groups II or III. Reduction of nitrates to nitrites was variable with different strains in both Groups I and II. Both strains of Group III were positive in respect to nitrate reduction.

Pathogenicity was tested subcutaneously in guinea pigs, intraperitoneally in mice and intravenously in rabbits. All three Groups appeared to be non-pathogenic for guinea pigs and mice by the routes tested. Groups I and II were non-pathogenic for rabbits intravenously. Group III, however, grown in Difco thioglycollate broth and inoculated in 0.5 c.c. amounts intravenously into rabbits was rapidly lethal in six out of eight rabbits.

None of the control rabbits inoculated with sterile broth reacted unfavourably. Death in the experimental animals occurred in periods ranging from as little as one hour up to about twelve hours. Post mortem findings included distention of the large bowel with liquefaction of bowel contents, engorgement of veins and sinusoids of the liver and edema of the lungs. A second series of animals was injected with either washed cells or with broth from which the cells had been removed by centrifugation. Four animals remained well after inoculation with washed cells but all four animals inoculated with supernatant died in the same way as animals previously inoculated.

February 5, 1952.

APPENDIX "G"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: Experimental fusospirochetal infection with mixtures of pure cultures

Grantee: Dr. J.B. Macdonald

Project No.: DR 35 Amount of Grant: $2,800

THE MOTILE ANAEROBES OF THE ORAL CAVITY

Cultural Requirements and Biochemical Activity

Temperature:

To test temperature optima and limits for growth of the 18 strains they were incubated in Difco Thioglycollate medium in Brewer's anaerobic jars at various temperatures - room temperature (approx. 20°C.), 27°, 37°, 40° and 45°C. Group I was incubated for 5 days and Groups II and III for 48 hours. The results are summarized in Table I. For all three Groups growth was as good or better at 37° than at any other temperature tested. The range of temperatures which consistently supported growth was narrower for Groups I and II (27° - 40°C.) than for Group III (20° - 45°C.).

Carbon Dioxide:

Growth of some anaerobes is enhanced in the presence of CO_2. The effect of various percentages of CO_2 in the atmosphere of hydrogen and nitrogen in which these motile anaerobes are grown was tested. Tubes of Difco Thioglycollate broth inoculated with the various strains were placed in Brewer's jars from which the air was exhausted sufficiently to obtain a 30% vacuum. Carbon dioxide was introduced into the jars in quantities sufficient to obtain final concentrations at atmospheric pressure of 5%, 10% or 20%. A fourth set of tubes received no added CO_2. Atmospheric pressure was restored by the introduction of hydrogen. Heated platinumized asbestos catalyzed the reduction of remaining atmospheric oxygen to water. The jars were incubated at 37° five days for Group I and 48 hours for Groups II and III. Results are summarized in Table II.

Growth of Group II was enhanced by the addition of as little as 5% CO_2. Greater amounts did not appear to increase the benefit. Groups I and III were not affected by the presence or absence of CO_2.

Oxygen:

Though none of these organisms will grow in atmospheric concentrations of oxygen it was felt worthwhile to assess their oxygen tolerance. This was done by heavily inoculating Noguchi tubes of melted Difco Thioglycollate medium 1% agar with the various strains.

These were then incubated aerobically at 37° for the same periods as used in the previous experiments. Group I failed to grow at all in the shake tubes.

Group II strains in 48 hours showed a band of heavy growth 1 to 2 mm. wide about 1 to 2.5 mm. below the surface. The band was sharply defined and paralleled the curve of the meniscus along its upper surface. A control tube incubated along with the shake tubes of Group II showed green colouration to a depth of 17 mm. indicating that oxygen tension to this depth was sufficient to oxidize the methylene blue in the medium. The tubes of Group II were re-incubated for three additional days and then re-examined. At this point floccular growth extended below the original band to the full depth of the tubes in all three strains. Group II thus appears to be microaerophilic, having a distinct preference for oxygen tensions slightly below atmospheric levels. This group, however, grows well though more slowly in the complete absence of oxygen.

Group III grew along the length of the tubes from the bottom to within a few millimeters of the surface (1 cm. for VB4 and 1.5 cm. for VB10).

Hydrogen Ion Concentration:

All 18 strains were incubated anaerobically at 37° in Difco thioglycollate broth adjusted to various hydrogen ion concentrations. The effect on growth is summarized in Table III. Group I incubated for 5 days grew at pH ranging from 6.0 to 8.6, but growth was poorer at the extremes of this range than in the centre. Group II grew best at 7.2 but light growth occurred from pH 6.5 to pH 8.6. It is important that the time of incubation for Group II was only two days because later studies on fermentation showed that these organisms in 5 days could lower the pH of a medium containing dextrose to 5.2 to 5.6 depending on the strain used. Group III strains grew over a wider range of hydrogen ion concentrations and indeed grew well and were motile from pH 5 to pH 8.6. Incubation time for Group III was 48 hours.

Biochemical Activity:

Fermentative power was tested by growth in a sugar-free thioglycollate broth prepared by Baltimore Biological Laboratories to which was added respectively 1% of dextrose, sucrose or mannite. Production of hydrogen sulphide in Groups I and II was tested in peptone iron agar tubes made by mixing equal parts of PSAFKE agar and Difco peptone iron agar. Group III strains were grown in a mixture of Difco thioglycollate medium and Difco peptone iron agar. Formation of indols was tested in Difco thioglycollate broth cultures by layering 4-5 drops of Ehrlich's reagent over the surface of the cultures after heating them to 55°C. Reduction of nitrate to nitrite was tested in BBL thioglycollate cultures containing 0.1% potassium nitrate. Presence of nitrite was tested by the method of Topley and Wilson using an acid solution of sulfanilamide followed by

ammonium sulfamate followed by x-naphthylamine. Positive controls using a culture of E. coli were run for the indols and hydrogen sulphide tests. The nitrite test was assessed quantitatively by the addition of sodium nitrite to BBL broth. It was found that approximately 0.0001% $NaNO_2$ gave a faint positive test. Results of biochemical activity tests are summarized in Table IV as determined after 5 days' incubation for Group I and 2 days' incubation for Groups II and III except in the case of the fermentation tests which were inoculated 5 days in all cases.

Of the three Groups, Group I was the least active biochemically. It was distinctly different than Groups II and III in that it failed to ferment sugars and it consistently produced hydrogen sulphide. Except for reduction of nitrate, the 12 strains within the Group appeared to be uniform. There were no clear cut differences between Groups II and III and again strains within each Group exhibited remarkable uniformity.

Pathogenicity:

The pathogenicity of these organisms was tested subcutaneously in guinea pigs, intraperitoneally in mice and intravenously in rabbits.

Guinea pigs:

Six strains of Group I cultivated in Difco thioglycollate broth for 5 days were inoculated in 1 c.c. amounts subcutaneously in the groin of young adult guinea pigs. Two animals were used for each strain and two animals were injected with uninoculated broth controls. The 3 strains of Group II were grown in PSAFKE broth for 3 days and inoculated into guinea pigs in 1 cc. amounts except for strain WT-OZ which grew heavily and from which only 0.75 cc. was inoculated. Two animals were used for each strain along with two controls which received sterile broth. The 2 strains of Group III were inoculated from 48 hour cultures in Difco thioglycollate broth. One cc. injections were used.

None of the animals developed demonstrable lesions over a period of two weeks.

Mice:

Five day cultures of Group I strains in PSAFKE (4 strains) of Difco thioglycollate broth (2 strains) were inoculated intraperitoneally into mice. Two mice were used for each strain and the amount injected was 0.2 cc. All Group II strains were inoculated in 0.2 cc. amounts from two day cultures in PSAFKE broth. Two mice were used for each strain. Two day cultures in Difco thioglycollate broth were used in the case of Group III strains and the injections were 0.2 cc. into two mice for each strain. Broth controls of each medium were inoculated in 0.2 cc. amounts into two mice.

The animals remained well until sacrificed in periods ranging from 5 days for Group I to two weeks for Groups II and III.

Rabbits:

Six strains of Group I 5-day cultures in Difco thioglycollate broth, three strains of Group II 2-day cultures in PSAFKE and two strains of Group III 2-day cultures in Difco thioglycollate were inoculated intravenously in 0.5 cc. amounts into normal rabbits. One rabbit was used for each strain. Controls of each type of broth were injected intravenously into one rabbit.

Rabbits inoculated with Groups I and II remained well as did the controls during a three week observation period following the infections. Of the two rabbits infected with Group III strains, one died within 12 hours. Post mortem examination revealed distension of the large bowel and liquefaction of bowel contents. Sections taken from the lung showed edema of the alveoli. Liver sections showed considerable engorgement of the central veins and sinusoids with blood.

Six more rabbits were injected as previously with Group III cultures, - three rabbits for each strain - along with three more normal controls. All the controls remained well but 5 of the 6 rabbits infected with cultures of VB4 or VB10 died. Of the total of 4 rabbits receiving VB4 intravenously 3 died and of the 4 receiving VB10, 3 died. Death in each instance was rapid - a maximum of 12 hours and as little as 1 hour. Death in each case was preceded by a rapid heart rate and laboured breathing. In one instance weakness of the hindquarters was observed immediately following injection. Post mortem examination in all cases revealed marked distension of the large bowel and in the relatively late death, liquefaction of bowel contents. It was observed that even when the animals were examined immediately after death there was no visible peristalsis. Organs appeared grossly normal but the trachea was observed to contain blood. No growth was obtained from heart's blood drawn sterilely and inoculated into Difco thioglycollate broth. Sections are under preparation from liver, spleen, kidney, adrenal and lung. In the one case examined histologically to date no changes were observed in kidney, spleen or adrenal.

It was noted that 48 hour cultures of VB4 and VB10 reduced the pH of the medium to 5.0 and it was felt advisable to inoculate controls of the broth adjusted to pH5. Four controls thus inoculated remained healthy.

The speed of death following inoculation suggested the presence in the culture of a toxic metabolite rather than infection as a cause of the lethality of these cultures for rabbits. To test this hypothesis a 48 hour culture of VB10 was centrifuged and the supernatant separated from the cells. The cells were then washed in saline and a heavy saline suspension was injected in 0.5 cc. amounts into 4 rabbits. The suspension was sub-cultured to test viability. Although the organisms were proven viable by the finding of good growth in the subculture the rabbits inoculated with them remained healthy. Four rabbits inoculated with the supernatant died within 2 to 12 hours in the same way and with similar post mortem findings as animals inoculated with whole cultures.

TABLE I

EFFECT OF VARIOUS TEMPERATURES ON GROWTH
OF ANAEROBIC MOTILE RODS

	Room Temp.	27°C.	37°C.	40°C.	45°C.
Group I	0	+	+	±	0 to ±
Group II	0 to ±	±	+	+	0 to ±
Group III	±	±	+	±	±

0 = no growth

± = light growth

+ = good growth

TABLE II

EFFECT OF CO_2 ON GROWTH OF ANAEROBIC MOTILE RODS

	No added CO_2	5% CO_2	10% CO_2	20% CO_2
Group I	+	+	+	not tested
Group II	±	+	+	+
Group III	+	+	+	not tested

± = light growth

\+ = good growth

TABLE II

EFFECT OF CO_2 ON GROWTH OF ANAEROBIC MOTILE RODS

	No added CO_2	5% CO_2	10% CO_2	20% CO_2
Group I	+	+	+	not tested
Group II	±	+	+	+
Group III	+	+	+	not tested

± = light growth

+ = good growth

TABLE III

EFFECT OF pH ON GROWTH OF ANAEROBIC MOTILE RODS

	pH								
	4.5	5.0	5.5	6.0	6.5	7.2	7.9	8.2	8.6
Group I	nt	nt	nt	±	+	+	+	nt	±
Group II	nt	nt	nt	0	0 to ±	+	±	±	±
Group III	±	+	+	+	≠	≠	+	≠	+

0 = no growth

± = light growth

\+ = good growth

≠ = heavy growth

nt = not tested

TABLE III

EFFECT OF pH ON GROWTH OF ANAEROBIC

MOTILE RODS

	pH								
	4.5	5.0	5.5	6.0	6.5	7.2	7.9	8.2	8.6
Group I	nt	nt	nt	±	+	+	+	nt	±
Group II	nt	nt	nt	0	0 to ±	+	±	±	±
Group III	±	+	+	+	+	+	+	+	+

0 = no growth

± = light growth

+ = good growth

+ = heavy growth

nt = not tested

TABLE IV

BIOCHEMICAL ACTIVITY OF ANAEROBIC MOTILE RODS

	Final pH					
	Dextrose	Sucrose	Mannite	Indole	H_2S	Nitrate Reduct.
Group I	No change	No change	No change	0	+	5 pos. 7 neg.
Group II	5.2-5.6	4.85-7.0	6.6-6.9	0	0	1 pos. 2 neg.
Group III	5.1	5.3	6.8	0	0	+

TABLE IV

BIOCHEMICAL ACTIVITY OF ANAEROBIC MOTILE RODS

		Final pH				
	Dextrose	Sucrose	Mannite	Indole	H_2S	Nitrate Reduct.
Group I	No change	No change	No change	0	+	5 pos. 7 neg.
Group II	5.2-5.6	4.85-7.0	6.6-6.9	0	0	1 pos. 2 neg.
Group III	5.1	5.3	6.8	0	0	+

APPENDIX "H"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: Chemical mechanisms in dental caries

Grantee: Dr. J.J. Rae

Project No. DR 1 Amount of Grant: $960

SUMMARY OF WORK

(1) A second paper on ammonia production in saliva by Ballantyne and Rae has been accepted for publication in the Journal of Dental Research in which it was shown that there is no relation between l.b.a. counts and ammonia production of saliva.

(2) An attempt was made to develop a rapid method for estimating caries-susceptibility by measuring the lactic acid production of saliva in the presence of glucose. No correlation between l.b.a. counts and lactic acid production was obtained and so the experiments were discontinued.

(3) Further work has been done on a bactericidal material obtained by treating tooth enamel with chloroform but the material has not yet been identified.

APPENDIX "B"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: Chemical mechanisms in dental caries

Grantee: Dr. A.J. Rae

Project No. DR'I Amount of Grant: $960

SUMMARY OF WORK

(1) A second paper on ammonia production in saliva by bacteria and Rae has been accepted for publication in the Journal of Dental Research in which it was shown that there is no relation between I.b.a. counts and ammonia production of saliva.

(2) An attempt was made to develop a rapid method for estimating caries-susceptibility by measuring the lactic acid production of saliva in the presence of glucose. No correlation between I.b.a. counts and lactic acid production was obtained and so the experiments were discontinued.

(3) Further work has been done on a bacteriolytic material obtained by freezing tooth enamel with chloroform but the material has not yet been identified.

APPENDIX "H"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: Chemical mechanisms in dental caries

Grantee: Dr. J.J. Rae (C.T. Clegg, assistant)

Project: DR 1 Amount of Grant: $960

Interim Report No. 13

The work covered in the period 1 February 1951 to 1 February, 1952, was subdivided into two parts.

(1) Ammonia Production in Saliva

A publication dealing with the second part of the work on ammonia production in saliva has been accepted by the Journal of Dental Research and reprints of this article should be available shortly. The results obtained showed that the activity of the ammonia-producing mechanism was unrelated to the l.b.a. count. The urease content of saliva was also unrelated to the l.b.a. count. Glucose was found to stimulate ammonia production in the presence of urea.

(2) Lactic Acid Production of Saliva

Since lactobacillus acidophilus produces lactic acid as one of the main products of its metabolism, it was thought that a chemical test for lactic acid production might serve as a means of estimating caries-immunity or susceptibility. If this were so, a more rapid method for measuring caries susceptibility than the 4-day l.b.a. count method would be available.

It was decided to use the colorimetric method proposed by S.B. Barker and W.H. Summerson in J.B.C. 138/535 in 1941, for the estimation of the micro amounts of lactic acid produced. The method finally evolved, was as follows:- To 3 ml. of fresh saliva, 3 ml. of 10% TCA solution was added. After 20 minutes the mixture was centrifuged and to 5 ml. of the centrifugate 1 ml. of 20% copper sulphate solution and 0.5 gm. of calcium hydroxide powder were added. The volume was adjusted with

water to 10 ml. After 30 minutes standing the mixture was centrifuged and 1 ml. of the supernatant liquid was transferred to a wide mouth test tube. 0.05 ml. of 4% $CuSO_4$ and 6 ml. of conc. H_2SO_4 were then added.

The tube was placed in boiling water for 5 minutes, cooled and 0.1 ml. of p-hydroxydiphenyl solution (1.5 gm. of p-hydroxydiphenyl in 100 ml. of 0.5% NaOH) added. After shaking, it was kept at 30° in a water bath for 30 minutes and then placed in boiling water for 90 seconds. After cooling theliquid was transferred to a colorimeter tube and compared with a standard lactic acid solution in a Klett-Summerson Photoelectric Colorimeter.

Using this method it was found that the lactic acid content of fresh saliva varied between 3 and 10 mg. % (mg. per 100 ml. of saliva).

Lactic acid production of saliva was determined in the following way:-

1 ml. of fresh saliva was mixed with 4 ml. of phosphate buffer solution (pH 5.0) and 1 ml. of 10% dextrose solution. The mixture was incubated at 37° for one hour and adjusted to a volume of exactly 15 ml. 1 ml. aliquot was used for lactic acid determination.

It was found that when dextrose was not added, no lactic acid was produced. Experiments have shown that the above method does not give a coloration in the presence of pyruvic acid (another end product of carbohydrate metabolism).

When the lactic acid production of salivas with various l.b.a. counts was determined, the results shown in the following table were obtained.

TABLE I

Relation Between l.b.a. Count and Lactic Acid Production

		Count	Lactic Acid Contained (mg. per 100 ml. Saliva)	Lactic Acid Produced in 1 hour (mg. per 100 ml. Saliva)
1	Saliva No. 9-1	60,000	4.2	20.6
2	" " 9-2	200,000	3.7	19.1
3	" " 9-3	300,000	2.7	45.0
4	" " 9-4	21,000	7.3	16.7
5	" " 9-5	30,000	3.0	61.0
6	" " 18-7	200,000	3.9	62.0
7	" " 25	0	9.0	45.7
8	" " 28-5	0	5.5	41.2

From these results it can be seen that there is no relationship between l.b.a. count and lactic acid production under these conditions. The fact that zero count salivas show significant lactic acid production could be explained by the fact that there are other acid-producing bacteria in saliva.

(3) An Inhibitory Substance for the Growth of l.b.a. in Tooth Enamel.

For some time now, we have been investigating a bactericidal material for l.b.a. prepared by shaking powdered tooth enamel with chloroform or bromoform. In summarizing the results to date, we have established the following:-

1. When ground tooth enamel was shaken with chloroform or bromoform for 30 minutes, the solvent decanted, and the enamel dried at 150°, the treated enamel (10 mg. in 4 ml. of culture) was found to have a bactericidal effect on l.b.a.

2. Calcium phosphate and bone meal, under similar treatment, had no such bactericidal effect.

3. When treated tooth enamel (shaken with chloroform) is extracted with alcohol, acetone or ether, the extract contained the bactericidal material.

4. On evaporation of the extract the residue is a yellowish green oily substance, which is very hygroscopic. Qualitative tests for elements showed that the residue contained no sulphur, nitrogen nor phosphorus but it does contain some halogen, which was probably chloride, since it gave negative tests for bromide and iodide.

5. When the extract was evaporated to dryness, the residue could be separated into a chloroform-soluble and chloroform-insoluble part. The chloroform-soluble part was found to be inactive, the chloroform-insoluble part, when dissolved in alcohol showed the bactericidal effect. The addition of alcoholic sodium hydroxide to the alcoholic solution gave a precipitate, which, when the pH was adjusted to neutrality with alcoholic citric acid solution, showed bactericidal effect.

6. Water, hot and cold, does not extract the inhibitory substance from the tooth enamel, but seems to inactivate it.

7. Samples of

(a) Chloroform-treated tooth enamel.

(b) Alcoholic solution of chloroform-insoluble part described in No. 5

(c) Precipitate obtained by adding NaOH to (b).

were sent to Eli Lilly Co., for testing against micro-organisms. Since the chloroform-treated tooth enamel (Sample (a)) was insoluble in water or alcohol, they dissolved the tooth enamel in dilute HCl before testing for bactericidal effect. They found no activity under these conditions, due no doubt, to hydrolysis of the active material.

The alcoholic solution (Sample (b)) was found to inhibit lactobacillus casei at dilutions as high as 1 in 200 in a broth test. This material was also found to have "a broad spectrum of activity" against a variety of micro-organisms.

Sample (c) was dissolved in acidic ethanol and was found to have slight activity against l. casei. There was not enough (4.2 mg.) for a broad screening test.

8. Such small amounts of the various fractions have been prepared that chemical tests have been impractical. In an attempt to overcome this difficulty teeth were collected from various sources and larger amounts of the alcoholic extract were prepared.

The work on this project is being continued.

APPENDIX "I"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: Part 1 - An investigation of the mechanism of repair of the supporting structures of the teeth;

Part 2 - Fusospirochetal infection and pre-existing inflammation.

Grantee: Dr. C.H. Best

Project No. DR 22 Amount of Grant: Part 1 $2300
Part 2 $1350

SUMMARY OF WORK

Part 1. Experimentally produced epithelialized periodontal pockets approximately 100 mm. in depth were prepared in dogs. These pockets were permitted to become infected so that smears taken from these pockets exhibited the typical pocket flora observed in periodontal disease. In the preparation and treatment of these pockets it was planned that, as far as possible, the situation created simulated the desired result following the so-called conservative treatment of periodontal disease.

It was found that under these conditions the experimental pockets were eliminated in part by the regeneration of the lost supporting structures. The epithelial attachment moved crownwise permitting reattachment by means of the laying down of new cementum on the denuded root surface. Most of the cases also showed degrees of regeneration of the alveolar process and periodontal membrane. The criteria were observations in the living animal and histologic sections.

Evidence will be presented that:

(1) The downgrowth of the oral peithelium does not present an insuperable barrier to regeneration of lost supporting structure.

(2) New cementum can be laid down on a denuded root surface that has been exposed for a considerable time to the oral fluid and oral flora.

(3) Removal of the epithelial attachment and the epithelium of the pocket wall is not necessary to permit cemental reattachment.

(4) The epithelial attachment can move occlusally as well as apically.

(5) Under suitable conditions the alveolar process will regenerate.

A detailed report of this study will be presented orally at the March meeting of the Committee

A paper reporting this phase of the investigation is being prepared for publication.

Part 2. Experimental fusospirochetal infection of laboratory animals provides a means of studying some of the factors which influence or may have a predisposing effect on fusospirochetal infection associated with periodontal disease.

The experimental infection is produced by parenteral injection of material from disease processes in the mouth which contains the fusospirochetal complex of organisms.

Studies of experimental fusospirochetal infection of normal guinea-pigs, rats and rabbits have been made and reported.

Studies of the effects of such factors as vitamin B and vitamin C deficiencies, trauma and products of inflammation have been made but they are not complete.

A more complete study of experimental fusospirochetal infection in normal and vitamin B deficient rats is reported in two papers which arebeing prepared for publication. Drafts of these are enclosed with this summary.

APPENDIX "I"

EXPERIMENTAL FUSOSPIROCHETAL INFECTION IN THE RAT

I Fusospirochetal Disease in Rats Maintained on a Balanced Diet.

II Fusospirochetal Disease in Rats Maintained on a Diet Deficient in the Vitamin B Complex.

Submitted by:-

D.C. O'Connell
Research Assistant
Banting and Best Department
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EXPERIMENTAL FUSOSPIROCHETAL INFECTION IN THE RAT

I. Fusospirochetal Disease in Rats Maintained On A Balanced Diet.

An investigation of the effect of certain factors upon experimental fusospirochetal infection of laboratory animals was facilitated when it was found possible to produce characteristic experimental lesions in normal albino rats (Wistar strain) by the subcutaneous injection of exudate from fusospirochetal lesions.

Although many investigators have reported the production of experimental fusospirochetal infection in guinea pigs, rabbits, mice, etc., very little use of the rat has been made for this purpose. Randall (1), working with rats maintained on a normal and on a rachitogenic diet, attempted without success, to produce lesions by the subgingival injection of material from Vincent's infection. He believed that the rat is not susceptible to Vincent's infection either when fed on a balanced ration or when its physical condition and resistance are greatly lowered by a rachitogenic diet. Clements (2) in studies on tropical ulcer reported successful production of cutaneous fusospirochetal ulcers in rats under certain special conditions. He maintained his animals on a diet similar to that of the New Guinea natives which he believed predisposed both the natives and the experimental animals to tropical ulcer. This diet was rich in carbohydrate, poor in protein and fat, and completely or partially deficient in Vitamin B_2. He concluded that for successful development of an ulcer, after inoculation with material from acute gingivitis, both depilation (due to faulty diet) and some vigorous trauma to the skin prior to injection were essential.

Many investigators have reported studies in relation to fusospirochetal disease, D.T. Smith (3) (4), Proske and Sayers (5), Davis and Pilot (6), Wallace, Wallace and Robertson (7), Tunnicliff and Klein (8), Tunnicliff and Hammond (9), etc., but the work of Rosebury and Foley (10) on experimental Vincent's infection cleared the way for the use of exudate from human lesions as a satisfactory substitute for pure cultures of the fusospirochetal complex which are technically difficult to obtain. They showed that the lesions produced in guinea pigs by the injection of fusospirochetal material from disease processes were characteristic and transmissible and that the exudate contained the organisms considered by other workers, D.T. Smith (3) (4), Proske and Sayers (5) to be essential.

For our purpose the use of fusospirochetal exudates and the lesions developed by them in guinea pigs has provided (as suggested by Rosebury and Foley) a very useful basis for study of some of the factors influencing experimental fusospirochetal infection. However, we

wished to study the influence that certain vitamin deficiences might have upon experimental fusospirochetal disease, and for this purpose the guinea pig was not satisfactory because it will not readily eat unbalanced synthetic diets. We hoped that the rat, which is much more omnivorous, would be useful in this type of investigation. Preliminary experiments showed that the rat is susceptible to infection with fusospirochetal exudate taken from lesions in the guinea pig and also from disease processes in the mouth.

This paper is a report of the results obtained when normal albino rats were injected with exudate from fusospirochetal lesions.

Experimental Fusospirochetal Infection in Guinea Pigs

Gingival scrapings from a human case of necrotic periodontitis were suspended in a small amount of infusion broth. This material in amounts of 0.3 cc. was inoculated subcutaneously into four guinea pigs. The remainder was preserved at the temperature of dry ice. When typical lesions developed in the animals, exudates were collected, portions frozen and some passed to other guinea pigs. This procedure was repeated at least three times by the method described by Rosebury and Foley (10). Careful scrutiny of the lesions produced, the exudates obtained and darkfield examinations of these exudates, led us to believe that they were satisfactory in spite of being derived from only third passage material. We considered them typical in all respects and 0.1 cc. of guinea pig lesion exudate injected subcutaneously into the groin of four guinea pigs produced abscesses with or without extension, which when aspirated between six and ten days after inoculations yielded several cubic centimeters of foul-smelling exudate, which was found by darkfield examination to contain spirochetes, fusiform bacilli, vibrios, cocci, and usually some other bacterial forms. No attempt has been made in this laboratory to grow the organisms in pure culture.

Preliminary Experiments

1. Twelve albino rats were inoculated subcutaneously in the groin area with fusospirochetal exudate from experimental lesions in guinea pigs. Six rats which received 1.0 cc. developed large necrotic abscesses. Of these two died 48 hours after injection. One was killed on the tenth day. The lesions in the remainder ruptured and drained and the animals survived.

The other six rats which received 0.1 cc. of the exudate failed to develop significant lesions. Autopsy after 17 days showed small localized thick walled abscesses.

Microscopic examination (darkfield) of the exudate taken from the rat lesions showed the typical bacterial flora including an exceptionally large number of tightly coiled spirochetes.

These findings suggested that larger amounts of experimental fusospirochetal exudate were required to produce significant lesions in rats. The smaller dose (0.1 cc.) used to transfer the infection in guinea pigs gave rise to only regressive lesions in the rats.

The early death of two of the rats might be attributed to the toxic property of fusospirochetal exudate mentioned by Graham (11).

2. Exudates from four clinical processes were injected into guinea pigs and rats. The animals were killed when the lesions in the guinea pigs were optimum. Consideration was given to possible species response variation.

(a) Gingival scrapings (preserved) from a case of necrotic periodontitis gave typical responses in guinea pigs but failed to produce significant lesions in 3 rats following the injection of 0.1 cc. of the material. Living fusospirochetal complex organisms were demonstrated in the lesions of four guinea pigs but in only one rat.

(b) A very dilute suspension of scrapings from a case of advanced simplex periodontitis failed to produce typical lesions in both guinea pigs and rats following the injection of 0.4 cc. of the material.

(c) A heavy suspension of gingival scrapings from a case of acute Vincent's infection injected into four rats in 0.4 cc. amounts and into four guinea pigs in 0.1 cc. amounts gave rise to typical lesions in the guinea pigs and to relatively large abscesses in three of the rats.

(d) To provide enough material for injection of a larger number of animals, the scrapings from six cases of necrotic periodontitis were pooled. Eight rats and six guinea pigs received 0.4 cc. each of the material. One half of each species was traumatized at the site of injection by movement of the needle.

All the guinea pigs and six of the rats developed significant lesions. Two rats showed small regressive abscesses.

The findings at autopsy of both species revealed no distinct difference in the lesions of the traumatized and untraumatized animals, but the lesions in the rats were less severe than those in the guinea pigs.

The results of these preliminary attempts to infect normal rats with fresh material suggested that gingival scrapings containing the fusospirochetal organisms were infective for the rats, but the lesions produced did not compare well with those in the guinea pigs.

3. The transmission of experimental fusospirochetal infection through rats was effected by the injection of guinea pig exudate

into rats, followed by injection of the rat exudate into both rats and guinea pigs.

(a) 0.2 cc. of rat exudate gave rise to significantly large necrotic lesions in four rats. Four guinea pigs received 0.1 cc. and subsequently developed large necrotic abscesses with subcutaneous extension; and four guinea pigs received 0.05 cc. and developed sizeable lesions.

The guinea pigs' lesions on aspiration yielded typical exudate which by microscopic examination (darkfield) resembles the picture one sees of an exudate which has been passed many more times. The fusospirochetal complex group of organisms predominated and the extraneous organisms (large open spiral spirochetes, large granular fusiform bacilli, aerobic contaminants, gas formers and filaments) which are often carried through several passages in the guinea pig, seemed to have been decreased in number.

(b) A sample of exudate from a fusospirochetal lesion in a rat, initiated by direct injection of gingival scrapings was passed (0.1 cc. subcutaneously) to two guinea pigs. On the 6th day after injection one guinea pig had a drained lesion. The other animal was killed the same day and autopsy revealed a 1.5 cm. abscess low in the groin from which 1.5 cc. of exudate was collected. By darkfield, this exudate was bacteriologically typical of one which had been passed many times through guinea pigs. The practical disappearance of the extraneous organisms from the exudate following passage through the rat is interesting and should hasten isolation of the fusospirochetal complex group of organisms by culture.

The results of these last two experiments would suggest that it is possible to transmit fusospirochetal infection from the guinea pig through the rat, to both guinea pigs and rats, and also to transmit the infection from the mouth, through the rat, to the guinea pig. The infective exudates certainly were not impaired but may have been enhanced by passage through the rat. Although these experiments should be repeated on many more animals, there is a suggestion that passage through the rat may eliminate several of the passages through the guinea pig which are usually carried out to rid the infective agent of many organisms which do not belong to the fusospirochetal complex. Rosebury et al (10, 12, 13, 14, 15, 16, 17) suggested that the infecting dose of gingival scrapings for guinea pigs is between 0.3 and 0.5 cc. and the infecting dose of passage exudate to be about 0.1 cc. It is obvious from the experiments carried out by Rosebury et al and by ourselves that the infecting dose varies considerably. It must be realized that as a rule insufficient material can be collected from one human case to inoculate a significant number of test animals. More material becomes available after passage through a guinea pig, but is this new inoculum strictly comparable with the original suspension of scrapings?

If one wishes to compare infectivity in guinea pigs and rats, some standard dose must be available. Although no such standard

exists, the data now available, suggest that the infecting dose for the rat is somewhat greater than that for the guinea pig.

Therefore, the purpose of the next experiment was to determine the amount of exudate, which had been carried several times through guinea pigs, required to infect a large percentage of inoculated rats.

A final passage of one strain of exudate through several guinea pigs yielded enough infective material to inoculate a significant number of rats. A comparison of infection in the rat and in the guinea pig cannot be made because the rat responds to inoculation of fusospirochetal material in much the same way as does the hamster, Shpuntoff and Rosebury (13). Early evacuation of lesions in the rat lowers fatality. Rosebury et al (10) have shown in the case of the guinea pig that two types of disease result from the subcutaneous inoculation of human mouth material and in a later paper Rosebury et al (16) (17) determined the LD 50 of the infective mixture. We did not attempt to establish such precise criteria at this time but only wanted to determine a range of infective dosage which would permit us to carry on other experiments using the rat as the test animal.

A fresh infective mixture* (later proved by culture to contain the essential organisms) which had been serially passed 8 times through the guinea pig was used as inoculum. Seven groups of rats each maintained on a balanced ration were injected in the groin region with 0.01, 0.05, 0.1, 0.2, 0.4, 0.6, and 0.8 cc., respectively, of the guinea pig exudate (diluted slightly). The animals were allowed to live for 3 weeks and at the end of this period all rats which remained alive were killed and autopsied. If any exudate was obtained microscopic (darkfield) examinations were made. Exudates from healing wounds or ulcers resulting from rupture of lesions prior to three weeks were also examined for the presence of fusospirochetal organisms. The results of this experiment appear in Table I.

Five control guinea pigs were inoculated subcutaneously at the same time with 0.1 cc. of the same material. One guinea pig died four days after inoculation and autopsy revealed the typical fatal extension commonly found in the guinea pig. The four remaining guinea pigs were killed eleven days after inoculation when their lesions were about to rupture. Autopsy of these animals showed the typical localized large abscesses in the groin with no extension. The bacteriological flora of all exudates were typically fusospirocheta.

Analysis of Results

Analysis of the results of this experiment shown in Table I reveals that as the dosage is increased the lesions become more

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* Exudate cultured by Dr. John B. Macdonald, Faculty of Dentistry, University of Toronto, Toronto, Canada.

severe, the time of evacuation of abscesses is shortened and the persistence of intact abscesses is decreased. Relatively small amounts of exudate never gave rise to very large abscesses. Usually the lesion was about 1 cm. in diameter and in about 50% of the cases these abscesses persisted for three weeks without evacuation.

The rats which received larger doses of exudate developed sizeable abscesses which tended to evacuate before two weeks. In a few animals large abscesses persisted intact for three weeks but these resulted from medium-sized doses.

In the first group, 8 animals developed small lesions, two of which evacuated early in the third week. Small regressive abscesses or nodules persisted in six of the rats until they were killed at 21 days. Lesions failed to develop in two of the animals in this group.

Nine rats in the second group developed fairly small abscesses. Of these, four evacuated during the third week, while five were intact on autopsy on the 21st day. One animal was found dead on the 8th day and autopsy showed a groin abscess and a small abscess in the dome of the liver.

All animals in the third group developed fairly small abscesses but in 5 cases evacuation occurred during the second week while four cases evacuated during the third week. One rat died on the 14th day from peritonitis, and evidence of peritoneal puncture was found indicating accidental intraperitoneal injection.

The rats in the fourth group developed larger abscesses than were found in the first groups and in three of these animals the lesions persisted at 2 cm. cavernous necrotic abscesses for 21 days. Abscesses in two rats evacuated early in the second week. Of the remaining five rats which died between the 6th and 12th days, two showed liver abscesses at autopsy in addition to the lesions in the subcutaneous tissues and three showed groin abscesses and peritonitis.

Only one rat in the 5th group had an abscess persisting for three weeks and in this case it was relatively small considering the dose given. Five animals had evacuation of large abscesses early in the second week but two of these had persistent multiple chronic abscesses in the mesenteries at sacrifice. Three of four rats in this group that died in the first week had peritonitis as well as large groin abscesses and one which was undoubtedly injected by the intraperitoneal route showed only peritoneal involvement when autopsied on the third day.

It is possible that following the large abscess formation, a bacteremia is set up which gives rise to small abscesses in other organs. This seems to have occurred in several cases. Evidence of mass infection with toxic manifestations was apparent in the sixth and seventh group of animals. Of those rats which received 0.6 cc. of exudate, four died very soon after inoculation, showing on autopsy

peritonitis and pleurisy, in addition to deposits of inoculum in the groin region. One animal which died on the ninth day was the only one in this whole experiment which showed the fatal extension type of lesion so commonly seen in the guinea pig. Again, four rats had abscesses which drained early in the second week and two of these had small multiple chronic abscesses elsewhere at autopsy after 21 days. One rat had only a 1 cm. abscess deep in the subcutaneous tissue of the groin in the retroperitoneal position. Five extra rats added to this group were positively injected subcutaneously with the same amount of comparable material. All of these developed large abscesses very early. One drained on the seventh day, two on the eleventh day, and two on the fifteenth day. Only one animal showed small multiple abscesses persisting for 21 days. The others had completely healed lesions when autopsied.

Of seven original rats in the seventh group, five were dead on the second day, and at autopsy all five showed deposits of inoculum in the groin, involvment of the peritoneum and fusospirochetal fluid in the chest cavities. The remaining two animals had large cavernous lesions which evacuated before seven days and one of these showed subsequent multiple small abscesses in the groin, in the mesenteries and an abscess in the dome of the liver. All of the five extra rats in this group developed large abscesses which drained early in the second week. Of these only one had a small abscess in the groin at autopsy. The others were completely negative.

In every case the fusospirochetal complex of organisms was demonstrated in the exudate from the lesions produced including the peritoneal and pleural fluids. Localized nodules and multiple chronic abscesses in the mesenteries also contained living fusospirochetal organisms.

Pathology

The type of lesion which develops in the rat after the subcutaneous injection of exudate containing the fusospirochetal complex of organisms varies greatly with the amount of inoculum.

With small amounts (0.01 - 0.05 cc.) there is a tendency to localization of the material from the outset which results in resorption if the injection is deep, or late evacuation of the abscess developed if the injection is shallow.

If the amount of inoculum is medium sized (0.2 - 0.4) a slight extension or enlargement of the abscess usually occurs resulting in abscesses about 1.5 - 2 cm. in diameter. Again these abscesses may evacuate early (within 14 days) or persist (14-21 days) depending upon the depth of injection of inoculum. Often a retroperitoneal spread occurs.

When relatively large doses are administered subcutaneously, several developments may take place. First of all a large cavernous raised abscess develops from the outset. This lesion will

evacuate as soon as it reaches the surface but this does not occur in all cases. Usually evacuation takes place within seven days, but we have observed many 4 cm. abscesses persisting for 21 days. In a very few cases these initially large abscesses did not evacuate but tended to spread forward and over the abdomen of the rat giving rise to a diffuse gangerous cellulitis which results in the death of the animal within one week.

In a few animals, infection of other organs in addition to abscess formation in the groin has been observed. Four fuso-spirochetal abscesses in the dome of the liver developed in rats inoculated with relatively small amounts of exudate (0.05 and 0.2 cc.) and six rats showed chronic abscess involvement of the mesenteries.

As a rule evacuation of lesions is followed by rapid healing although fusospirochetal organisms can be demonstrated in the draining exudate for several days after rupture. No chronic ulcers have been observed in any of the rats.

By daily examination of rats following subcutaneous injection of fusospirochetal exudates from disease processes the course and type of lesion obtainable in rats has been recorded so that now the extent of a lesion can to a degree be anticipated. Slight localized swelling without marked erythema is first noticed. This is followed in two to four days by either a smaller indurated area which will persist or disappear within two weeks, or a larger soft abscess which will usually evacuate within two weeks. If the lesion is going to rupture, the skin becomes ischemic and gangerous a day or so before evacuation takes place. The fatal extension type of lesion in the rat cannot readily be detected because the area destroyed by the advancing infection is not thickly walled off and consequently is not palpable. Fusospirochetal peritonitis does not usually develop in rats injected subcutaneously but there is some evidence to suggest that bacteremias do occur and in some animals where there was some doubt as to the route of inoculation, rapid spread of the infection throughout the peritoneum did occur bringing about the early death of the animal, and in other animals whose subcutaneous lesions evacuated, small multiple nodular abscesses were found in other organs.

These experiments have shown that experimental fusospirochetal infection in normal rats can be readily induced and transmitted from animal to animal. An analysis of the results obtained in normal rats indicate that the rat can be used in place of the guinea pig in experiments dealing with nutritional deficiencies, and the effect which these deficiencies might have upon experimental fuso-spirochetal infection.

The information thus collected by experimentation with fuso-spirochetal infection in the normal rat has been put to use in other experimental infection studies. Such a study is one carried out to determine the effects of a dietary deficiency of the Vitamin B complex on experimental fusospirochetal infection.

Summary

Experimental fusospirochetal infection of white rats has rarely been attempted. In this report, two such attempts are reviewed. In each case the test rats were not normal.

Results of preliminary experimentation on normal rats using fusospirochetal material from various sources as inocula are given. Comparison of experimental fusospirochetal infection and transmission of this infection has been made in guinea pigs and rats.

An attempt has been made to establish a range of infecting dose for the normal rat so that studies of the relationship between physiologic and pathologic conditions and experimental fusospirochetal infection might be carried out.

The pathology of experimental fusospirochetal infection in normal rats has been outlined.

TABLE I

RESULTS OF INOCULATION OF WHITE RATS WITH FOSOSPIROCHETAL EXUDATE

Dosage in cc.	No. of rats	Route of subcutaneous	Inoculation Intraperitoneal	Fatal infection	Localized abscesses in groin	Abscesses evacuated before 21 days. 1st wk.	2nd wk.	3rd wk.	Abscesses not evacuated in 21 days	Other autopsy findings	Negative in 21 days
0.01	10	10	0	0	8	--	--	2	6	one draining ulcer	2
0.05	10	10	0	1 LA	10	--	--	4	5	--	0
0.1	10	9	1	1 P	10	--	5	4	-	one SN deep one SA groin	0
0.2	10	7	3	5 2LA 3P	7	--	2	--	3	--	0
0.4	10	6	4	4 4P	6	--	5	--	1	2 small multiple abscesses in mesent.	0
0.6	15	6+5	4	5 4P+ Pl. 1FE	11	1	4+4	--	1+1	3 mesent. abscesses 1 LA	0
0.8	12	2+5	5	5 P+Pl	7	2	5	--	--	1 SA groin 1 SA mesent.	0

LA = Liver abscess
P = Peritonitis
SN = Small nodule
SA = Small abscess
Pl = Pleurisy
FE = Fatal extension

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EXPERIMENTAL FUSOSPIROCHETAL INFECTION IN THE RAT

II. Fusospirochetal Disease in Rats Maintained on a Diet Deficient in the Vitamin B complex.

Introduction

The first paper in this series reported fusospirochetal infection in normal rats by the subcutaneous injection of exudate from fusospirochetal lesions. Since it was found possible to produce characteristic experimental lesions in the normal rat, an investigation of the effect of certain vitamin deficiences on experimental fusospirochetal infection was facilitated. This paper is a report on the effect of a Vitamin B complex deficiency on experimental fusospirochetal disease in rats.

There are many references in the literature to the effect that deficiences of some of the factors of the Vitamin B complex have upon the metabolism of the animal body (ref.) 1, 2, 3, 4, 5, 6. Deficiencies of some of these factors have specific effects such as degenerative changes in the tissues, and reduced cellular infiltration in inflammation. A study of the effect of a vitamin B deficiency on fusospirochetal infection seemed warranted.

In order to circumvent specific effects due to dietary lack of certain factors of the Vitamin B complex, it was decided to consider a dietary deficiency of the B complex as a whole in a study of the effect of vitamin B deficiency on experimental fusospirochetal infection.

Procedure

One hundred and seventeen rats weighing between 125-150 gms. were placed in individual cages and divided among 12 groups by weight. These consisted of 9 groups of 9 rats each, two groups of 10 rats each, and one group of 16 animals.

43 animals (9,9 and 16) were maintained on a diet deficient in Vitamin B complex and choline, and fed ad libitum.

37 animals (9,9,9 and 10) were pair-fed with the deficient diet group and maintained on a balanced diet.

37 animals (9,9,9 and 10) were maintained on a balanced diet and fed ad libitum.

DIETS

Ingredients	Balanced Diet	Deficient Diet
Gelatin	2.0 %	2.0 %
Casein	12.0	12.0
Fibrin	1.0	1.0
Sucrose	68.65	71.0
Beef fat	5.0	5.0
Corn oil	2.0	2.0
Cellu flour	2.0	2.0
Salts	5.0	5.0
P.D.W. vitamins	2.0	0.0
Cod liver oil	0.015	0.015
tocopherol ac.	0.010	0.010
Choline cl.	0.35	0.0

When the animals on the deficient diet showed the characteristic symptoms of a vitamin B deficiency such as loss of weight, hair and vigor, all of the rats except those set aside as controls were injected with fusospirochetal exudate. This was after the animals had been on the deficient diet for 25 days.

Inoculum and Method of Injection

The exudate used in this experiment was taken from a subcutaneous abscess in a guinea pig which developed after injection of 0.1 cc. of 12th passage guinea pig exudate. The material originally came from a human case of necrotic periodontitis. This exudate was examined by both microscopic and cultural means and contained all of the essential members of the fusospirochetal complex of organisms. The rats were injected subcutaneously (with minimal trauma) in the lower abdomen using a No. 19 needle.

Dosage

The dosage of exudate varied within the three main groups of rats and the inocula were brought up to constant volume of 0.1 cc. by dilution with normal saline.

Deficient Group

9 rats received 0.01 cc. exudate

9 " " 0.05 "

9 " " 0.1 "

Pair-fed Group

9 rats received 0.01 cc. exudate

9 " " 0.05 "

9 " " 0.1 "

Normal Control Group

9 rats received 0.01 cc. exudate

9 " " 0.05 "

9 " " 0.1 "

Animal Death

The deficient control (14) and deficient injected rats lived an average of 31 days on the inadequate diet. Only 6 days elapsed on the average, between the time of infection and death in the injected animals. It is evident that the effects of the dietary deficiency rather than of the injection were responsible for the death of these animals.

The pair-fed rats maintained on the balanced diet did not die with the exception of 3 rats which were injected, and 4 rats which were not. The injected animals were killed at intervals of 7 and 11 days. The control rats were sacrificed on the 35th day of the experiment.

The dietary control-group of rats fed ad libitum on the good diet all lived with the exception of one animal which died ten days after injection. The remainder of injected animals were sacrificed at intervals of 7 and 11 days.

The uninjected control animals were sacrificed on the 35th day of the experiment.

Pathological Findings

All animals in this experiment were autopsied. Particular attention was paid to the general condition of the animal, the weight of the animal at death and to lesions if any in the injected rats.

Exudates in lesions encountered were examined microscopically by darkfield illumination. The uninjected deficient rats which ate less than 5 gms. of food per day for the last 17 days of the experiment were found to be very light in weight and emaciated. No gross pathologic changes were noted.

The uninjected pair-fed rats were also emaciated but not so severly as were the deficient animals. Those which died during the experiment showed evidence of intercurrent respiratory infection.

The uninjected control animals on the good diet were found to be fat, normal rats. Gain in weight was very marked (average = 66gm.)

The deficient animals lost an average of 56 gms. during the experimental period. The pair-fed rats lost 46 gms. on the average.

Analysis of lesions found in injected animals

Analysis of fusospirochetal infection induced by injection of infective exudate is based on the size and type of lesion which results. Results of experimentation with normal rats showed that: as the dosage is increased, the lesions become more severe, the time for evacuation of abscesses is shortened and the persistence of intact abscesses is decreased.

Because early death of the deficient rats necessitated the killing of some of the other animals so that a comparison of lesions could be made, and because about two weeks time will pass before abscesses resulting from relatively small doses will rupture and drain, consideration cannot be given in this experiment to evacuation of lesions. Only size and type of lesion were considered and these were recorded on a + or - basis.

Criteria

-	Small fibrinous nodule
+-	Pea-sized localized nodule without exudate
+	Pea-sized localized nodule with exudate
++	An abscess 1 cm. in diameter with frank exudate
+++	Any abscess up to 3 cm. in diameter
++++	Any abscess larger than 3 cm. or a lesion which has any great extension from the site.

All exudates encountered were found to contain the fusospirochetal complex of organisms and did not differ in any way from exudate collected from abscesses in control guinea pigs which were injected with 0.1 cc. amounts of the same material at the same time. The pathological

findings are recorded in Tables 2, 3 and 4, along with other pertinent data. The sums of the plus signs designating the size and type of lesions found in all of the injected rats indicate that the deficient animals which received only 0.01 cc. of exudate were more susceptible to infection than the other two groups which received the same infecting dose. The difference in pathological findings between the three groups of animals receiving the other two doses (larger doses) are not significant.

Rats	Dosage of exudate and sums of lesion designation		
	0.01 cc.	0.05	0.1
Deficient	13+	18+	28+
Pair-fed	7+	20+	24+
Normal control	4+	19+	24+

Conclusion

The results of this experiment indicate that rats maintained on a diet deficient in the B vitamins are predisposed (or more susceptible) to experimental infection with fusospirochetal exudate. This predisposition to infection is demonstrated by injection of a very small amount (0.01 cc.) of fusospirochetal exudate into deficient, pair-fed and normal rats. When 5 times or 10 times the minimal dose was injected into corresponding groups of animals, the slight differences in the overwhelming lesions produced were not significant.

Summary

Rats maintained on a diet deficient in the B vitamins are predisposed to subsequent experimental fusospirochetal infection.

This predisposition to experimental infection was demonstrated in only those animals which were injected with very small amounts of fusospirochetal exudates.

The effect of a Vitamin B complex deficiency on experimental fusospirochetal infection in rats is aggravation of the lesions.

ble I Vitamin B Complex Deficient Group of Rats

Injected Rats

Animal	Death in days after injection	Amount of exudate	Death wt.	Severity of lesion	Pathology
501	5	0.01 cc.	74 gms.	+	Pea-sized abscess (hem.)
502	7	"	83 "	++	1 cm. subcutaneous abscess
503	6	"	82 "	+	Pea-sized abscess
504	5	"	84 "	-	No lesion
505	3	"	87 "	+	Pea-sized lesion (hem. lung)
506	7	"	76 "	++	1 cm. abscess flat
507	10	"	80 "	++	1 cm. abscess flat
508	7	"	80 "	+++	Flat spreading abscess
509	7	"	96 "	+	Pea-sized abscess
511	3	0.05 cc.	83 "	-	No lesion
512	3	"	94 "	+	Pea-sized lesion
513	10	"	74 "	+++	Large flat abscess
514	6	"	82 "	+++	Large abscess
515	8	"	88 "	+++	Large abscess
516	7	"	87 "	++	Enlarging abscess
517	4	"	92 "	+++	Large spreading abscess
518	3	"	92 "	+++	1 cm. abscess - extension
519	1	"	78 "	-	No lesion
520	4	0.1 cc.	80 "	+++	Large necrotic abscess
521	7	"	85 "	+++	Large necrotic abscess
522	9	"	72 "	+++	Large necrotic abscess
523	9	"	80 "	+++	Large necrotic abscess
524	7	"	82 "	+++	Large necrotic abscess
526	7	"	86 "	+++	Enlarging abscess
527	8	"	84 "	++++	Very large abscess spread
528	4	"	80 "	+++	Large flat abscess
529	6	"	80 "	+++	Large necrotic abscess

Uninjected Rats

	Days on diet	Death wt. in gms.	Pathology
530	28	80	No gross pathological findings. All rats were emaciated and unthrifty in appearance.
531	31	74	
532	32	90	
533	29	82	
534	30	86	
535	30	88	
536	36	76	
537	31	76	
538	28	80	
539	29	86	
540	27	83	
541	33	90	
542	34	86	
543	41	95	

Average starting weight for group 139 grams.
Average death weight for group 83 "
Average loss in weight for group 56 "

TABLE II — Pair-fed Control Group of Rats

Animal	Sacrificed days after injection	Amount of exudate	Death wt.	Severity of lesion	Pathology
544	7	0.01 cc.	112 gms.	+-	Pea-sized nodule no pus
545	7	"	102 "	+-	Pea-sized nodule no pus
546	7	"	108 "	-	Small Fibrotic area
547	7	"	106 "	+	1 cm. indurated nodule
548	7	"	112 "	+-	Pea-sized nodule no pus
549	11	"	104 "	+	Pea-sized abscess
550	11	"	106 "	+	Pea-sized abscess
551	11	"	90 "	++	1 cm. abscess
552	11	"	122 "	++	1 cm. abscess
553	7	0.05 cc.	112 "	++	1 cm. abscess
554	7	"	94 "	+++	2 cm. cavernous abscess
555	7	"	104 "	++	1 cm. abscess
556	7	"	115 "	+++	1 x 3 cm. abscess raised
557	4 D	"	84 "	-	Deposit of inoculum only
558	11	"	96 "	+++	2 cm. abscess drained
559	11	"	102 "	+++	2 cm. abscess draining pus
560	11	"	100 "	++	1 cm. abscess drained
562	11	"	90 "	++	1.5 cm. abscess draining
563	7	0.1 cc.	104 "	+++	2 cm. cavernous abscess
564	7	"	100 "	+++	1 large and 2 small abscess
565	7	"	98 "	+++	1 large " 1 " deep "
567	7	"	114 "	++++	Large 4 x 3 x 2 raised "
568	11	"	105 "	+++	2 cm. abscess spreading
569	11	"	102 "	++	1 cm. abscess
570	11	"	96 "	+	Pea-sized abscess
571	11	"	102 "	++	1 cm. abscess
572	11	"	82 "	+++	3 cm. necrotic abscess

Uninjected Rats

	Days on diet	Death wt.	Pathology
561	21 D	110	No gross pathological findings in this group. All rats were under-nourished and somewhat unthrifty.
566	22 D	98	
573	35 S	98	
574	34 D	96	
575	35 S	97	
576	35 S	104	
577	35 S	87	
578	35 S	101	
579	35 S	106	
580	35 S	108	

Average starting weight for group 148 grams
Average death weight for group 102 "
Average loss in weight for group 46 "

D - Died
S - Sacrificed

BLE III — Normal Control Group of Rats

nimal	Sacrificed days after injection	Amount of exudate	Death wt.	Severity of lesion	Pathology
581	7	0.01 cc.	200 gms.	+-	2 small hard nodules
582	7	"	194 "	+	Pea-sized abscess
583	7	"	178 "	-	Small fibrinous nodule
584	7	"	202 "	-	" " "
585	7	"	190 "	+	Less than 1 cm. abscess
586	11	"	202 "	+-	2 small hard nodules
587	11	"	214 "	-	1 small nodule
588	11	"	198 "	+	Less than 1 cm. abscess
589	11	"	188 "	+	" " " "
590	7	0.05 cc.	192 "	++	1.5 x 2 cm. raised abscess
591	7	"	186 "	++	1.5 cm. marble abscess
592	7	"	191 "	++	2 less than 1 cm. abscesses
593	7	"	202 "	++++	5 x 2 cm. spreading abscess
594	7	"	174 "	++	1 cm. abscess
595	11	"	282 "	++	" "
596	11	"	192 "	+	Small drained abscess
597	11	"	199 "	++	1 cm. abscess
598	11	"	174 "	++	Fairly large drained abscess
599	7	0.1 cc.	174 "	++	Two 1 cm. abscesses
600	7	"	180 "	+++	2 x 3 cm. abscess
601	7	"	176 "	+++	" " "
602	7	"	212 "	+++	" " "
603	7	"	194 "	++	1.5 cm. raised abscess
604	10 D	"	186 "	++	1 cm. abscess (interc. infect.
605	11	"	170 "	++++	Extension to axilla (drain)
606	11	"	192 "	+++	2 cm. drained lesion
607	11	"	236 "	++	1.5 cm. raised abscess

Uninjected Rats

	Days on diet	Death wt.	Pathology
608	35 S	200	No pathological findings in
609	35 S	189	this group.
610	35 S	196	
611	35 S	218	
612	35 S	203	
613	35 S	203	
614	35 S	186	
615	35 S	200	
616	35 S	211	
617	35 S	211	

Average starting weight for group 131 grams
Average death weight for group 197 "
Average gain in weight for group 66 "

D - Died
S - Sacrificed

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APPENDIX "J"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: Further studies in the electromyography of the head, face and neck.

Grantee: Dr. R.E. Moyers

Project No.: DR 37 Amount of Grant: $ 960

SUMMARY OF WORK

The work in electromyography this past year has been directed towards "An Analysis of Myo-functional Orthodontic Therapy (Rogers)."

It was first shown by Rogers* that muscle exercises could aid in the correction of certain dento-facial deformities. Subsequently it was discovered** that abnormal reflex patterns of muscle contraction might be etiologic to dento-facial deformity.

During this past year and a half a series of patients undergoing routine orthodontic appliance therapy were studied electromyographically. The sample was divided so that some patients received myo-functional and appliance therapy, others the appliances alone. Electromyograms were secured routinely prior to, during, and at the completion of treatment. These records were supplemented by standardized cephalometric roentgenograms taken at the same time.

The findings may be summarized as follows:

1. Myo-functional therapy exhibits a more marked effect on the muscles of young children than adolescents or adults.

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* Rogers, Alfred P. Evolution, development and application of myo-functional therapy in orthodontics. Amer. Jour. Ortho. 25:1-19, 1939.

** Moyers, R.E. Temporomandibular muscle contraction patterns in Angle Class II div. 1 malocclusions: an electromyographic analysis. Amer. Jour. Ortho. 35:837-857, 1949.

Moyers, R.E. An electromyographic analysis of certain muscles involved in temporomandibular movement. Amer. Jour. Ortho. 36:481-515, 1950.

2. Beneficial changes in the muscle contraction pattern are produced routinely by myo-functional therapy where the primary etiologic site is dental (e.g. malposed teeth on normal jaw bones, tooth interferences to normal occlusion, etc.)

3. Where the primary etiologic factor is within the facial skeleton (e.g. micrognathia, mandibular hypertrophy, or gross osseous dysplasia), myo-functional therapy is of little use.

4. The physiologic rationale of myo-functional therapy is an overt attempt to upset the afferent arms of the reflex arc maintaining mandibular posture and educate the neuro-muscular system to establish a new postural reflex.

5. The electromyographic record of the first set of exercises provides an excellentprognosis of the results which may be expected.

This work was carried out in the Electroencephalographic Laboratory of the Toronto General Hospital. Co-operating in the project was Dr. John Scott, Toronto General Hospital electro-encephalographer, and Dr. G.P. Copeland who is writing his M.Sc.(Dent.) degree in this area. The technician employed was Mr. Walter Kuczyk.

February 8, 1952

APPENDIX "K"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: Development of a method for determining fluorine by means of a photo-electric colorimeter.

Grantee: Dr. O.J. Walker

Project No.: DR 28 Amount of Grant: $1,000

SUMMARY OF WORK

This project has been nearly completed.

Results may be summarized under three headings.

(a) The determination of fluorides in water making use of the bleaching effect of fluorides on the lake formed by zirconyl salts and sodium alizarin sulfonate has been studied critically, and a superior procedure has been worked out in which rapid analyses can be carried out using a photoelectric colorimeter.

(b) A second method for determining fluorides has been studied. This is based on the bleaching by fluorides of the aluminum-hematoxylin lake. Conditions under which satisfactory analyses can be obtained have been ascertained and a rapid photoelectric colorimetric method has been developed. This method is less affected by sulfates and phosphates than are other methods.

(c) The molybdenum blue method for determining small amounts of phosphates has been examined from the standpoint of conditions that will lead to consistent results both in the presence and absence of fluorides. A satisfactory procedure has been developed.

Three papers, one on each of the above subjects, have been prepared and submitted for publication.

February 2, 1952.

APPENDIX "K"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: Development of a method for determining fluorine by means of a photo-electric colorimeter.

Grantee: Dr. O.J. Walker

Project No.: DR 28 Amount of Grant: $1,000

(a) Determination of fluorides in water by the zirconyl-sodium alizarin sulfonate method.

This phase of the Project has been fully discussed in previous reports. This investigation has now been completed and a paper containing the results has been submitted for publication in the Canadian Journal of Chemistry. The Abstract reads as follows:

"Fluorides in water may be determined rapidly in a photo-electric colorimeter. The method described is based on the decomposition by fluorides of the red lake formed by a zirconyl salt and sodium alizarin sulfonate using similar conditions to those proposed by Scott. In the photoelectric colorimeter, use is made of a 15 cm. absorption cell and a 515 mμ monochromatic filter. In this method there is no interference from phosphates up to 3 ppm. Sulfates interfere when over 150 ppm., but the interference is progressive so that a correction can be easily applied."

(b) Determination of phosphates in water photometrically by a modification of the molybdenum blue method.

A method for the rapid determination of small amounts of phosphates is of prime importance when determining fluorides in natural waters and other materials, as phosphates interfere greatly in all existing colorimetric methods, both visual and instrumental, for the determination of fluorides.

Work on this phase was commenced in 1949-50 and preliminary statements have been made in previous reports. Much of the work has been repeated and added to. The results have been embodied in a paper presented for publication to the Canadian Journal of Chemistry. Many of the features of the proposed method are included in this abstract.

"A modification of the methods for determining small amounts of phosphates by the molybdenum blue reaction has been proposed. In this method a photoelectric colorimeter utilizing a 15 cm. absorption cell and a 440 mμ monochromatic filter is used and care is taken that the reagents are always added in the same order, that a constant pH of 4.3 is maintained and that readings are made from 1½ to 2 hours after the reagents have been added. The method has given accurate results up to 6 ppm. When fluorides are less than 3 ppm. there is no interference, but above this amount there is interference even though large amounts of borates are present."

(c) Determination of fluorides in water by the aluminum-hematoxylin method.

The method for determination of fluorides in (a) has the disadvantage of being interfered with by phosphates to a considerable extent and by large amounts of sulfates. It was our hope to investigate other methods in order to learn if any one could be found or developed in which interference is less. This work was done partly in the winter of 1950-51 and in the summer of 1951.

The highly colored lakes formed by aluminum salts and hematoxylin and zirconyl salts and hematoxylin have been known for some time. The zirconyl-hematoxylin lake is bleached by small amounts of fluorides and this effect has been used in developing methods for determining small amounts of fluorides in water by such investigators as Gad and Naumann(1), Jendrassik and Papp (3), andJendrassik and Dippold (4). The corresponding aluminum-hematoxylin lake has been made the basis of a method for determining small amounts of aluminum (2). It is also bleached by fluoride ion and a colorimetric method for small amounts of fluoride in water has been described by Okuno (5,6). The present authors were unable to duplicate the results of Jendrassik and co-workers with the zirconyl lake and also those of Okuno with the aluminum lake. Much of the difficulty in working with these lakes is the instability of the hematoxylin. It seems to undergo oxidation resulting in modification of the color and intensity of the metallic lake.

Many modifications were made in the zirconium-hematoxylin method but none of these gave satisfactory results. However, it has been possible to propose a satisfactory method for determining small amounts of fluorides in water based on the bleaching of the aluminum-hematoxylin lake. This method differs widely from the one proposed by Okuno as can be seen by considering the following details.

Reagents and Materials

From a stock solution containing 2.21 gm. of C.P. sodium fluoride per liter, 10 ml. were taken and diluted to 1 liter with distilled water, (1 ml. =0.01 mg. F^-).

The standard solution of aluminum salt was prepared from C.P. aluminum sulfate and contained 0.05 mg. of aluminum per ml.

The preparation of hematoxylin solution presented considerable difficulties to previous workers as it seemed to oxidize over a period of time and thus the composition of the lake was not a constant. Jendrassik and Dippold (4) after making up the hematoxylin solution passed a stream of air through it for a period of time. In the experience of the present authors there was much uncertainty in this operation so an oxidized form was obtained by using a definite quantity of hydrogen peroxide. Better results were also obtained by recrystallizing hematoxylin, Eastman Kodak Practical, three times from water to which had been added a little sodium sulfite. It was also found necessary to alter the method of making up the solution. The precedure finally chosen is as follows. Fifty ml. of water and 5 ml. of 0.1N HCl are placed in a 250 ml. flask and heated to 60°C on a water bath. To this is added 0.1 gm. of recrystallized hematoxylin. Shake and continue heating until dissolved. While still warm, add 0.02N NaOH until the solution just turns red. Now add 3 ml. of 3% hydrogen peroxide followed by 100 ml. of 1% acetic acid. Allow to stand one hour before using.

One also needs a saturated solution of sodium bicarbonate and a 1:2 acetic acid solution.

The reagent or "indicator" is made up by placing 100 ml. of the standard aluminum salt solution in a liter flask and then adding 850 ml. of distilled water and 25 ml. of saturated sodium bicarbonate solution. After mixing there is added 15 ml. of the oxidized hematoxylin solution. Mix again and allow to stand for one hour. Now add 10 ml. of 1:2 acetic acid and shake. Allow to stand for 48 hours before using. A fresh indicator solution should be prepared each week. The lake is purple in color.

Experimental

Absorption spectra of hematoxylin and of the purple complex

Figure 1 contains the absorption spectrum of hematoxylin, A, as well as that of the purple lake, B. The maximum absorption of the purple lake occurs at about 550mμ and the absorption due to hematoxylin is small at this wave length. Therefore, when fluorides were determined with the photoelectric colorimeter the 550 mμ light filter was the proper one to use.

Calibration curves

The calibration curve for fluoride is obtained by making up a series of solutions, 100 ml. in volume, using from 0.0 to 14 ml. of the standard sodium fluoride solution containing from 0 to 0.14 mg. of fluoride and corresponding to 0 to 1.4 ppm. To each is added 10 ml. of the indicator. Allow to stand for a definite period of time, transfer to the 15 cm. absorption cell and read in the colorimeter. Per cent transmission as determined is plotted against ppm. of fluoride ion (Figure 2). Distilled water is used as a blank (Transmission = 100).

Effect of time

(a) Time seems to have some effect on the behavior of an indicator. Series of determinations were carried out using portions of the same indicator that had been made up for 2, 6, 9, and 26 days. The results are shown in Table I. The per cent transmission obtained for different amounts of fluorine did not differ much for the first 9 days but when it had been kept for 26 days, the per cent transmission, at similar fluorine content, differed widely from those obtained previously.

Table I

Effect of Time on Behavior of Indicator

	% Transmission			
ppm. F^-	2 days	6 days	9 days	26 days
0.0	9.1	9.5	9.6	17.6
0.2	13.4	12.9	13.2	22.4
0.4	24.9	22.8	21.9	27.6
0.6	42.0	35.9	35.3	40.0
0.8	55.9	49.6	46.3	45.0
1.0	69.3	61.0	55.8	51.5
1.4	84.5	86.5	85.3	85.0

(b) After the reagents have been added to the sample the extent to which the lake is bleached by fluoride depends on the time that elapses before the measurement is made. In Figure 2 are presented two calibration curves prepared from measurements taken after standing for 2 hours (A) and 4 hours (B) respectively. There is not a great deal of difference so that either time of standing may be used. Experience has indicated that better duplication can be obtained if the longer time is selected so that the 4 hour period is recommended.

Effect of pH

A survey of the color of the lake and its fading by fluorides was made at different pH's. It was found that at pH's above 6 (6.3, 7.3 and 9.1) thepurple color was very intense at first, but on standing changed to orange. There was no bleaching on the addition of fluorides. At pH's from 5.0 to 6.0 there was a precipitation of aluminum hydroxide. Below a pH of 4.2 the color of the indicator is too faint and there is very little change in color on the addition of fluorides. Satisfactory color development and bleaching took place at a pH of 4.6.

Several buffers were investigated. Okuno (5.6) had used ammonium carbonate as a buffer for regulating pH. In these investigations this reagent led to an unstable indicator and it was not possible to obtain results that checked. Even at a pH of 4.6 the indicator turned yellow on standing. The use of sodium succinate was investigated but indicators containing it failed to develop color. Satisfactory results were obtained when sodium bicarbonate was used. It was an easy matter to arrive at a pH of 4.6 and the color development was quite satisfactory. It was therefore used in all determinations reported.

Effect of Sulfates

Sulfates interfere with all visual and photometric methods for determining fluorides. Table II contains the results for fluoride obtained with sulfates up to 1200 ppm. when from 0 to 1.4 ppm. of fluoride were present. There is some interference at a sulfate content of 200 ppm. At 1200 ppm. the interference is such that a correction of 0.2 ppm. of fluoride is necessary. This is somewhat less than found by Walker and Finlay for the zirconyl-sodium alizarin sulfonate method.

Table II.

Effect of Sulfates on the Determination of Fluorides

F^- Present ppm.	ppm. $SO_4^=$ Added: 100	200	500	800	1000	2000
	ppm. F^- Found					
0.0	0.0	0.1	0.2	0.3	0.3	0.3
0.2	0.2	0.3	0.4	0.4	0.4	0.5
0.4	0.4	0.5	0.5	0.6	0.6	0.6
0.6	0.6	0.6	0.7	0.7	0.7	0.8
0.8	0.8	0.8	0.9	0.9	1.0	1.0
1.0	1.0	1.2	1.2	1.1	1.2	1.2
1.2			1.3	1.3		1.4
1.4	1.4	1.5				

Effect of Phosphates

The interference by phosphates follows an irregular pattern as can be seen from Table III. Each value is the average of several determinations. With no fluorides present the interference is equivalent to a correction of 0.1 ppm. of fluoride with as little as 0.2 ppm. of phosphate, and rises to 0.4 ppm. for a phosphate content of 8 ppm. With higher fluoride concentrations, 0.4 to 1.4 ppm., the correction is negligible up to at least 8 ppm. of phosphate. It can thus be seen that phosphates interfere less than in other colorimetric and photometric methods for determining fluorides in water.

Table III.

Effect of Phosphates on the Determination of Fluorides

ppm F^- Added	ppm. $PO_4^=$: 0.2	0.5	1.0	3.0	5.0	8.0
	ppm. F^- Found					
0.0	0.1	0.2	0.2	0.4	0.4	0.5
0.2	0.3	0.3	0.3	0.4	0.5	0.4
0.4	0.4	0.4	0.4	0.4	0.4	0.5
0.6	0.6	0.6	0.6	0.6	0.6	0.6
0.8	0.8	0.8	0.8	0.8	0.8	0.8
1.0	1.0	0.9	0.8	0.9	1.0	1.0
1.2	1.2	1.2	1.2	1.2	1.1	1.1
1.4	1.3	1.4	1.3	1.4	1.3	1.3

The determination of fluorides in water can be carried out satisfactorily by a photometric method involving the bleaching effect of fluoride ion on the aluminum-hematoxylin lake provided that the hematoxylin is partly oxidized previously and that the pH is closely regulated. The method has the advantage that sulfates and phosphates interfere to a smaller extent than in other photometric and colorimetric methods. It can be used satisfactorily in the examination of water supplies that naturally contain fluorides as well as for those that have been fluoridated. The limit of fluoride content that can be measured is 1.5 ppm. but with larger concentrations smaller portions of water are taken and diluted to a definite volume with distilled water.

This phase is now completed and a paper on the subject has been submitted to the journal of Analytical Chemistry of the American Chemical Society for publication.

(d) Some study has been devoted to the destruction of the organic matter in teeth in order to try out a more rapid distillation procedure which will be satisfactory for removing large amounts of phosphates prior to determination of fluorides by one of the photoelectric colorimetric methods. This project is thus in two steps (1) Digestion, (2) Distillation. We have not yet been successful in the first step so this investigation is what we would like to undertake in this coming year. Step 2 has received considerable study and we feel that this part of the problem has been solved.

The bulk of the experimental work carried out during the past year has been done by Mrs. Margaret Price. In my opinion her work has been very creditable.

References to Part C

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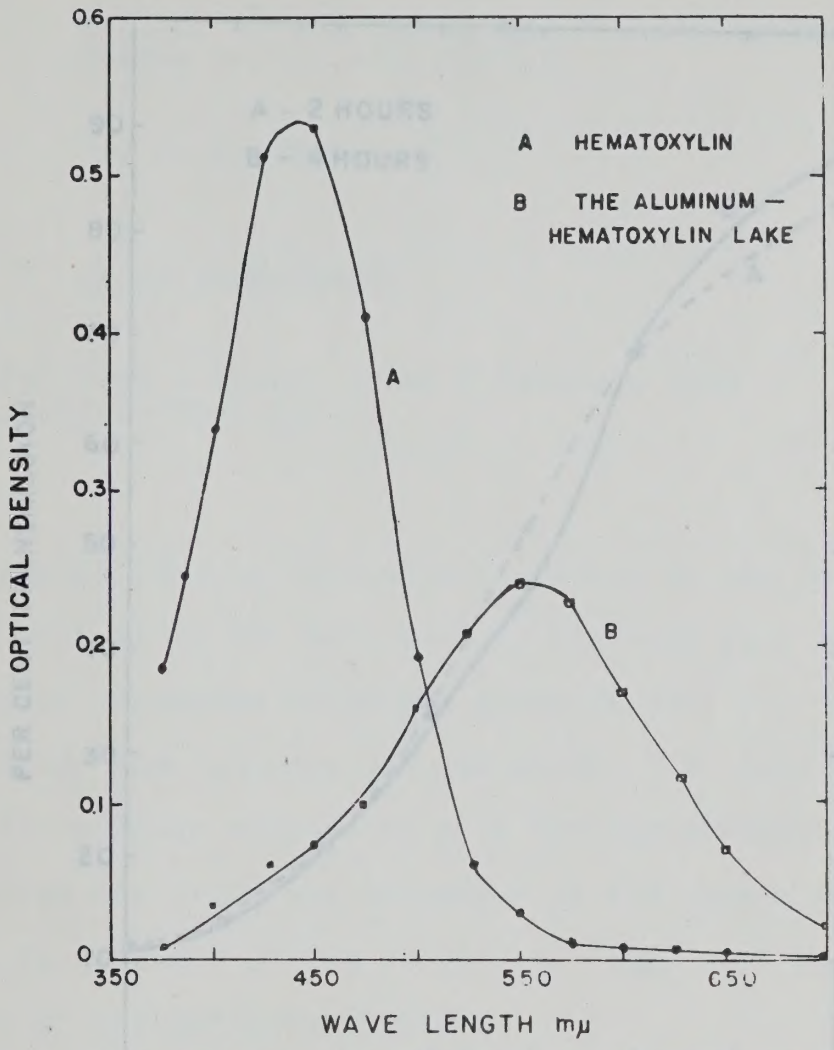

Figure 1 ABSORPTION SPECTRA OF HEMATOXYLIN AND OF THE ALUMINUM – HEMATOXYLIN LAKE

Figure 1 ABSORPTION SPECTRA OF HEMATOXYLIN AND OF THE ALUMINUM – HEMATOXYLIN LAKE

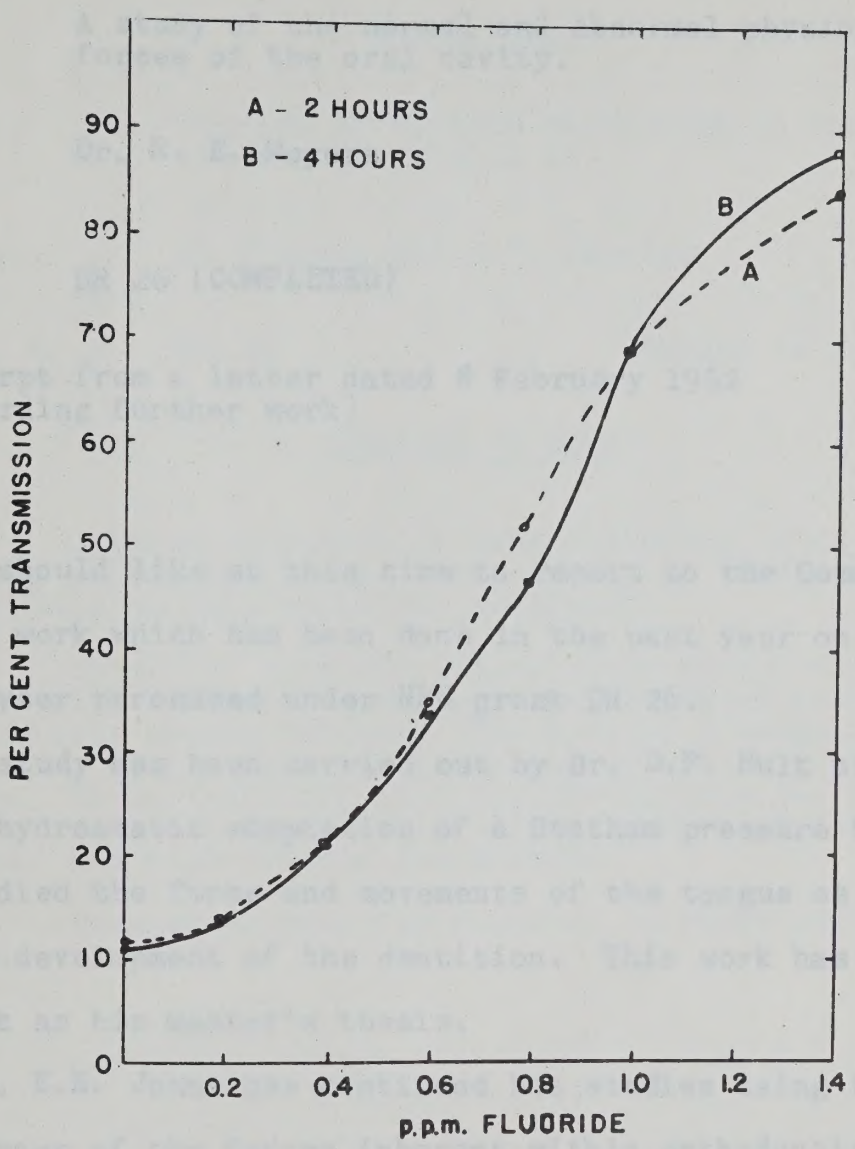

Figure 2 EFFECT OF TIME ON PER CENT TRANSMISSION

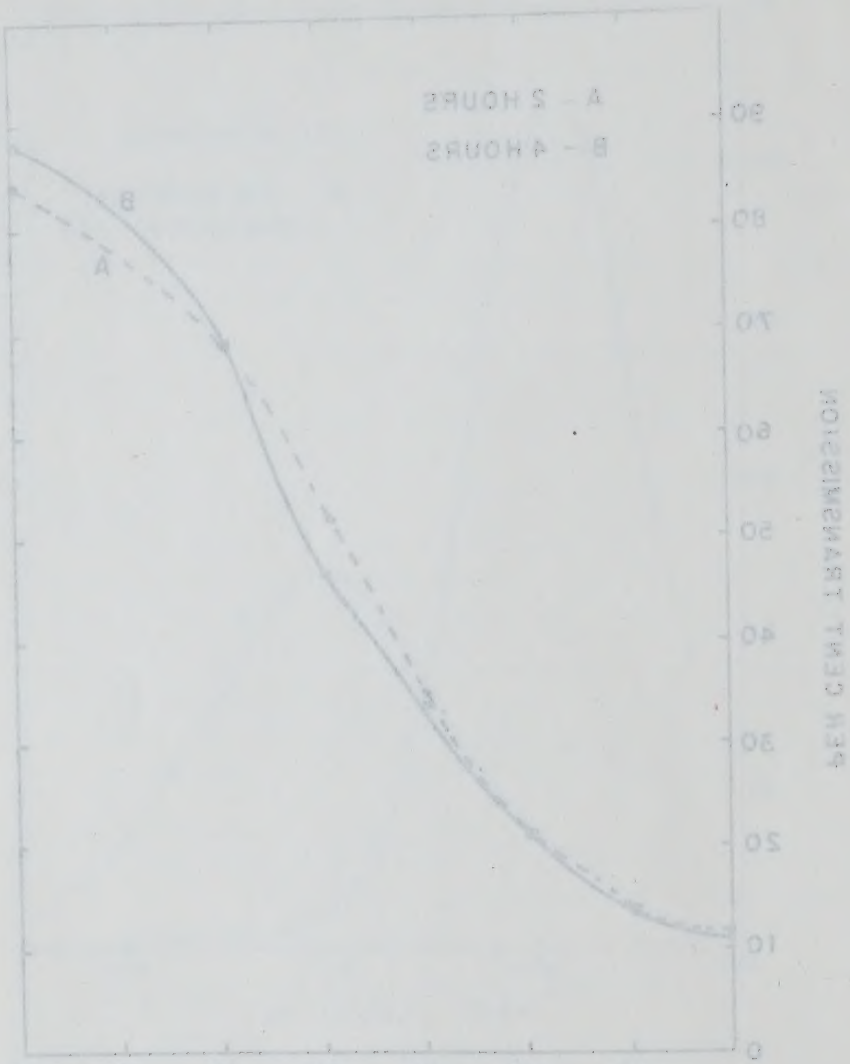

Figure 2 EFFECT OF TIME ON PER CENT TRANSMISSION

APPENDIX "L"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1952

Subject: A study of the normal and abnormal physiologic forces of the oral cavity.

Grantee: Dr. R. E. Moyers

Project No.: DR 26 (COMPLETED)

(Excerpt from a letter dated 8 February 1952 regarding further work)

.....

I should like at this time to report to the Committee on further work which has been done in the past year on the strain-gauge analyzer purchased under NRC grant DR 26.

A study has been carried out by Dr. D.P. Hult using an ingenious hydrostatic adaptation of a Statham pressure transducer. He has studied the force and movements of the tongue as they affect the development of the dentition. This work has been carried out as his master's thesis.

Dr. E.E. Johns has continued his studies using the strain-gauge analyzer of the forces inherent within orthodontic appliances during therapeutic tooth movements. His findings are to be presented as a part of a paper to be read on the program of the American Association of Orthodontists in St. Louis.

We will deposit with the Committee copies of all three of the papers mentioned when they are completed, as well as reprints after publication.

.....

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Appendix "M"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: Studies of Calcium Metabolism in Tooth with the aid of Calcium 45.

Grantee: A. D'Iorio and J.P. Lussier

Project No.: DR 34 Amount of Grant: $1,200

SUMMARY OF WORK

Calcium retention in bone following intraperitoneal injection of Calcium 45 has been studied as previously reported. Further data on this topic are presented.

Under the same nutritional conditions, animals are injected with radioactive calcium, and the blood collected for radioactivity determinations. The rate of disappearance from circulating fluids can thus be estimated with a good approximation. It is indicated that hypoproteinemia due to a protein-free diet does not appreciably differ from the data recorded in normal and high-protein fed animals.

Further progress is reported on other parts of the project.

February 9, 1952.

APPENDIX "M"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1952

Subject: PART I:- The turnover of radiocalcium in the blood of normal and protein deficient rat

Grantee: Dr. A. D'Iorio and Dr. J.P. Lussier

Project No.:DR 34 Amount of Grant: $1200

Introduction

It was indicated in a previous work (1) that the calcium turnover is reduced in calcified tissues when animals are kept on a protein-free diet. In order to evaluate the blood supply as a contributing factor to these changes, it is proposed to follow the rate of disappearance of radiocalcium when animals are fed either on normal, protein-free or high-protein diets. Works performed with radioactive calcium and reported up to now do not include much material related to calcemia. Harrison and Harrison (2) have followed the entrance of Ca^{45} from the gastro-intestinal tract into the circulating blood in the rachitic and treated rats. Linke et al (3) have just recorded serum values during a continuous intraperitoneal administration of Ca^{45}. Norris and Kisieleski (4) have established the behaviour of radioactive Ca, Sr and Ra in the blood after intravenous injections. Govaerts (5) has computed the simultaneous appearance of Ca^{45} in urine and its disappearance from the blood in the dogs until 6 hours after administration.

Experiments:

Newly weaned rats are reared the following diets: diet (N) containing 18% casein and diet (S) 50%; diet (D) being protein-free. The animals are fed their respective diet for a week prior to injection. $Ca^{45}Cl_2$ solution having a radioactivity of 7.56 x 10^4/cpm/ml is given intraperitoneously at the rate of 0.5 ml per 100 gms of body weight. Each group is subdivided in six subgroups according to the time elapsed between the injection and the blood withdrawal. Table I shows data related to interval between injection and blood withdrawal, number of animals, initial and final mean weight and blood sample volumes. Blood samples withdrawn by heart puncture, are transferred to a centrifuge tube containing 0.2 ml of herapin solution. After centrifugation, an aliquot of serum is transferred to 10 ml graduated flask. 5 ml of CCl_3 COOH 10% is added and the volume completed with distilled water.

An aliquot of this solution is pipetted to another centrifuge tube and one ml of $CaCO_3$ solution containing 50 mgm/ml is added. The calcium is precipitated the normal way by adjusting to pH 5.5-6.0, with NH_4OH and adding a suitable volume of saturated ammonium oxalate. The precipitate is centrifuged, washed with NH_4OH 2%, dried, weighed and uniformely spread on an aluminum sample tray for radioactivity measurement.

Results:

Considering that the blood volume is nearly 6.7% of the total body weight (7)(6), and that the hematocrit for the rat is approximately 50% of the whole blood volume, it was assumed that values multiplied by 3.35 would thus give a good idea of the total radioactivity in the plasma per hundred grams of animal body weight. The ratio of this radioactivity to the one injected indicates the proportion of Ca^{45} in the circulating blood.

The calculating formula has been used:

$$\frac{\text{Radioactivity recovered}}{\text{Radioactivity injected}} = \frac{[(\text{cpm} \times 0.0177)+1] \times Ft \times 73 \times F\ dil \times Fh \times 3.35}{w}$$

where cpm = reading from the G.M. Counter (less back ground)

w = weight of Ca oxalate used for the reading

73 = " " " " in the whole sample

Ft = decay factor

(cpm x 0.0177) + 1 = correction for self absorption

$F\ dil = \frac{10}{v_1 \times v_2}$ v_1 = volume of plasma used

v_2 = aliquot per radioactivity reading

Fh = correction for added heparin

3.35 = 6.7 x .5, to report values of serum per 100 gms of body weight.

Comments:

The rate at which calcium 45 escapes the systemic circulation is very rapid. In less than 40 minutes, Ca^{45} is in equilibrium with the extra-cellular fluids, if we consider with Gamble (8) that the extra-cellular fluid volume is 3 times as great as blood volume. At this time most of the radioactivity is still in the liquid phase.

The adsorption phenomena are taking place appreciably after the first half hour up to the tenth hour, then the equilibrium can be said to be established between the solid and liquid phases. About 1% only of the radioactivity can be recovered in the blood after 24 hours. This amount slowly decreases in the blood as the desorption takes place between the bone salts and the plasma. Although some values are still missing (N_6, S_5 and S_6) it is clearly demonstrated that hypoproteinemia following a protein-free diet does not appreciably influence the turnover of calcium in the plasma. This is an unexpected finding if we consider that a rough estimation indicates a 25% reduction in the total plasma proteins.

Values of serum protein per 100 ml of serum

N	D	S
5.8	4.5	5.7

The excess of casein in the diet is also without any noticeable effect.

By the findings plotted in Fig. 1, it may be induced that the protein level in the diet has no effect in the turnover of calcium in the blood.

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PART II:- Turnover of calcium 45 in the lond bones of the rat as influenced by the dietary protein level

Introduction:

In a previous report (1), early data on the effect of the protein level in the diet upon the calcium retention in the bones have been presented. It has been assumed that a protein-free diet by depriving the long bones from their protein reserve induces a slower turnover of radiocalcium in the epiphyses.

Methods and Results:

1.- Bones:

Experimental procedures and methods have been described in a preceeding report (1). Data treated by correcting factors on animal weight and dosage indicate in the deficient groups, that the calcium retention slightly decreased at the beginning, last longer than the normal and the high-protein fed animals. This is obvious in the tibia, the humerus and the carcass as illustrated in Table III. The behaviour of S subgroups in relation to calcium retention is somewhat different from the normals during the rapid desorption phase i.e. in the first half of the curves (Fig. 11).

The high protein level in the diet seems to have a reducing effect on the calcium uptake as shown by Sherman (2). The fixed calcium is released from bone, especially epiphyses at a slower rate. However, as this action is not sustained for the whole duration of the experiment, no interpretation can be proposed until more support can be given to this fact. We have made the hypothesis that the stop of growth induced by a protein fast would be a way of estimating how far the turnover of calcium in the bone would be different from the one present in normal growing animals.

Comparing data on the first day between D_1 and N_1, we see that the retention is about 70% larger in the normal. Extrapolating on the curves of Fig. I from the first point (24 hours) to approximately eight hours: i.e. at the time where the bone is in equilibrium with the highest circulating radioactivity we see that N_1 practically doubles D_1. After 12 days, the ratio $\frac{N_5}{N_5 - D_5}$ indicates that the turnover rate of D_5 is about half of N_5. Thus it appears that differences between N and D groups can only be attributed to the decreased turnover rate. The effect of growth is completely hidden by the adsorption - desorption phenomena.

2.-Excreta:

Available data on excreted radiocalcium (Table IV) require some additions before definite conclusions can be drawn.

3.-Teeth:

About the method for separating enamel from dentin, we were informed after personal communication with Dr. R.S. Manly, that Dr. Gilda is working on such a method which has not yet been described in any paper. Moreover, we are actually awaiting a sectioning machine which will readily give 50μ thick sections. Some material already embedded in bioplastic will be then processed.

4.-In vitro experiments:

For the same reason no in vitro experiments were undertaken. As soon as the sectioning machine will enable us to prepare adequate specimen, the teeth so graciously supplied by Dr. H. K. Brown will be used.

REFERENCES

1. D'Iorio A., and J.P. Lussier, Report to the Associate Committee on Dental Research, September 1951.

2. Sherman H.C., et al. Proc. Nat. Acad. Sc. 33; No 12 p.356, December 1947.

TABLE I

DIET	N					D					S				
Subgroup	No. of animals	Initial mean weight (gm)	Final mean weight (gm)	Blood pool (ml)	Interval (hr)	No. of animals	Initial mean weight (gm)	Final mean weight (gm)	Blood pool (ml)	Interval (hr)	No. of animals	Initial mean weight (gm)	Final mean weight (gm)	Blood pool (ml)	Interval (hr)
1	4	47	61	6.0	0.6 - 75 *	5	50	46	5.8	0.75 - 0.9 *	4	51	59	5.1	1.1 - 1.2*
2	5	48	63	4.0	1.6 - 1.8x	5	47	42	4.7	1.8 - 2.0 x	5	49	66	5.9	2.0 - 2.2x
3	10	42	57	10.3	4.5	10	44	39	6.3	4.5	9	45	63	11.2	4.5
4	5	38	57	7.5	7.75	3	35	35	2.9	8.0	4	39	60	6.3	8.0
5	5	47	58	2.0	18.0	5	46	40	3.1	18.0	4	47	55	6.1	19.5
6	4	44	63	8.1	23.5	5	47	42	2.4	22.0	4	44	60	7.6	23.5

*N_1 35-45 *D_1 45-55 *S_1 65-70

xN_1 100-110 xD_2 110-120 xS_2 120-130

TABLE II

PERCENTAGE OF THE RADIOACTIVITY FOUND IN THE PLASMA

Groups	cpm/mg at 0 thickness x Ft (1)	(1)x 73 (2)	F.dil.	(2)x F (3)	F h.	(3)x F h. (4)	(4)x 3.35 (5)	Radioactivity recovered / Radioactivity injected
N_1	8.1	590	4	2360	1.01	2505	8380	0.22
N_2	2.4	173	6.25	1081	1.10	1189	4020	0.10
N_3	10.0	730	0.4	292	1.06	311	1210	0.032
N_4	0.68	49.6	5	248	1.05	260	872	0.023
N_5	0.1	7.3	10	73	1.18	86	288	0.01
D_1	6.8	496.0	3.12	1547	1.06	1640	5500	0.14
D_2	2.3	168	4.54	763	1.07	816	2730	0.072
D_3	5.8	427	0.42	179	1.08	194	650	0.017
D_4	0.5	36.5	7.7	281	1.11	312	1039	0.028
D_5	0.5	36.5	10	365	1.13	412	1385	0.036
D_6	0.2	14.6	10	146	1.25	182	610	0.016
S_1	5.0	365	4	1460	1.07	1562	5240	0.14
S_2	3.8	277	4.54	1258	1.07	1346	4520	0.12
S_3	12.0	876	0.4	350	1.06	371	1242	0.033
S_4	1.3	94.9	5	475	1.06	503	1685	0.045

TABLE IV

RADIOACTIVITY DUE TO THE RADIOACTIVITY INDUCED IN THE PLANES

Groups	opaq/sq cm x Pl (1)	(1)x 73 (2)	P.dil.	(2)x P (3)	P n.	(3)x P n. (4)	(4)x 3.35 (5)	Radioactivity recovered Radioactivity injected
N_1	8.1	590	4	2360	1.03	2415	8340	0.22
N_2	2.4	173	6.25	1081	1.10	1189	4020	0.10
N_3	10.0	730	0.4	292	1.06	311	1210	0.032
N_4	0.68	49.6	5	248	1.05	260	872	0.023
N_5	0.1	7.3	10	73	1.18	86	288	0.01
D_1	6.8	496.0	3.12	1547	1.06	1640	5500	0.14
D_2	2.3	168	4.54	763	1.07	816	2730	0.072
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D_4	0.5	36.5	7.7	281	1.11	312	1039	0.028
D_5	0.5	36.5	10	365	1.13	412	1385	0.036
D_6	0.2	14.6	10	146	1.25	182	610	0.016
S_1	5.0	365	4	1460	1.07	1562	5240	0.14
S_2	3.8	277	4.54	1258	1.07	1346	4520	0.12
S_3	12.0	876	0.4	350	1.06	371	1242	0.033
S_4	1.3	94.9	5	475	1.04	505	1695	0.045

TABLE III

SPECIFIC ACTIVITY IN CARCASS AND IN DIFFERENT REGIONS OF TIBIA AND HUMERUS.

DIET	N								D						S						
Subgroup	TIBIA			HUMERUS			CARC.		TIBIA		HUMERUS			CARC.	TIBIA			HUMERUS			CARC.
	E	M	D	E	M	D		E	M	D	E	M	D		E	M	D	E	M	D	
1	673	306	110	603	225	114	256	378	229	65	282	158	71	156	539	233	91	404	150	74	245
2	312	226	109	342	219	118	178	324	220	87	255	280	74	172	442	266	81	392	215	106	207
3	294	266	125	259	215	131	192	244	228	76	200	155	59	137	300	233	97	300	188	110	195
5	126	132	110	120	128	92	121	190	194	70	186	163	73	135	120	142	89	114	91	95	117
6	89	-	108	75	110	76	111	-	-	-	-	-	-	-	65	85	82	64	75	69	69

| DIAT. | X | | |

TABLE IV

RADIOACTIVITY FOUND IN EXCRETA (cpm per 100 gms OF BODY WEIGHT)

Subgroup	N	D	S
1	-	72	176
2	376	93	95
3	57	41	76
5	94	40	30
6	16	-	12

TABLE IV

RADIOACTIVITY I DUMD IN BOUNDRY (ECOR DET JOI/gms OF BODY WEIGHT)

Subgroup	R	J	S
1	-	72	176
2	376	83	95
3	57	44	76
5	94	40	30
6	16	-	12

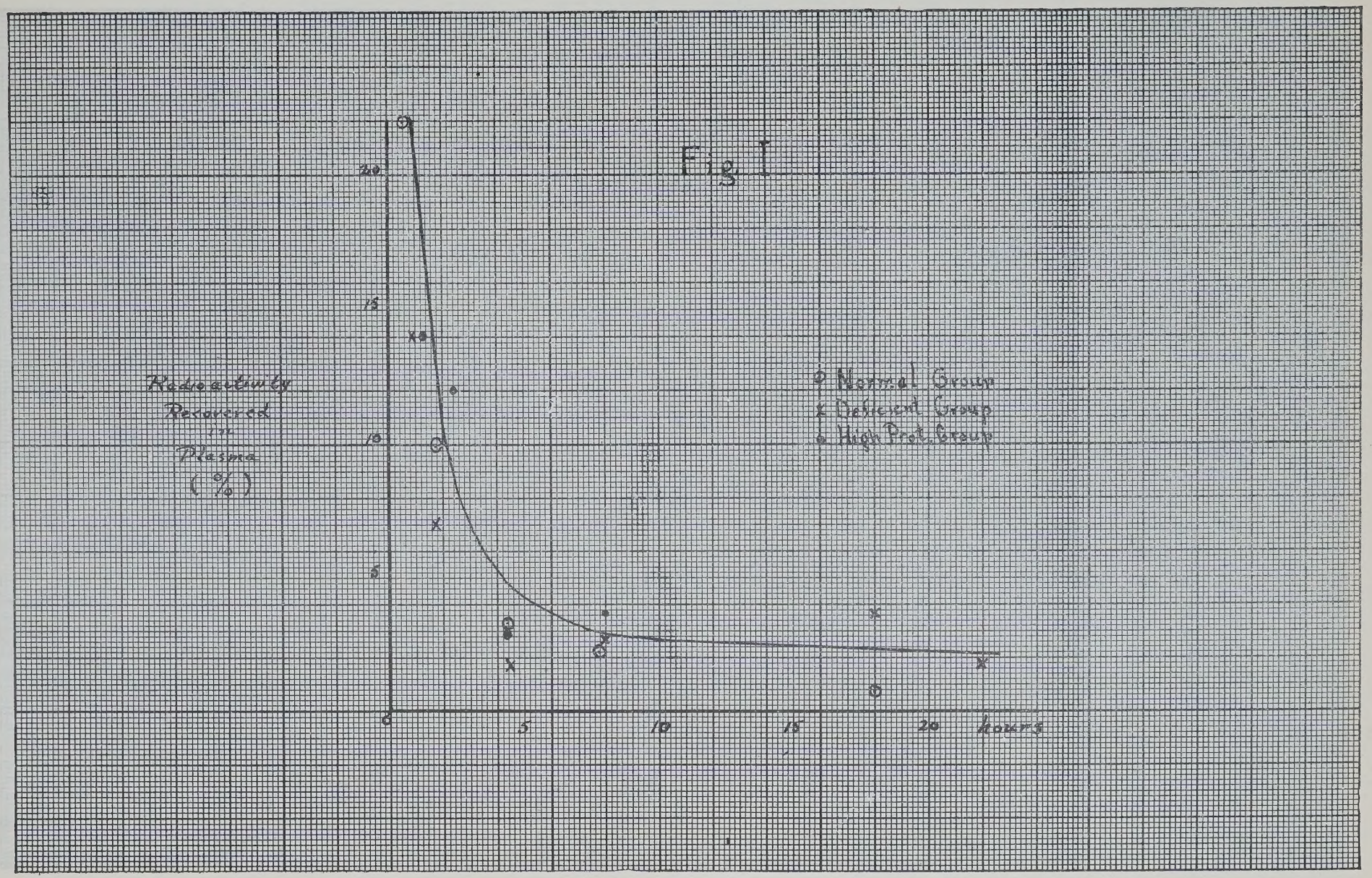
Fig I
Radioactivity Recovered in Plasma (%)
20
15
10
5
0
5
10
15
20
hours
o Normal Group
x Deficient Group
o High Protein Group

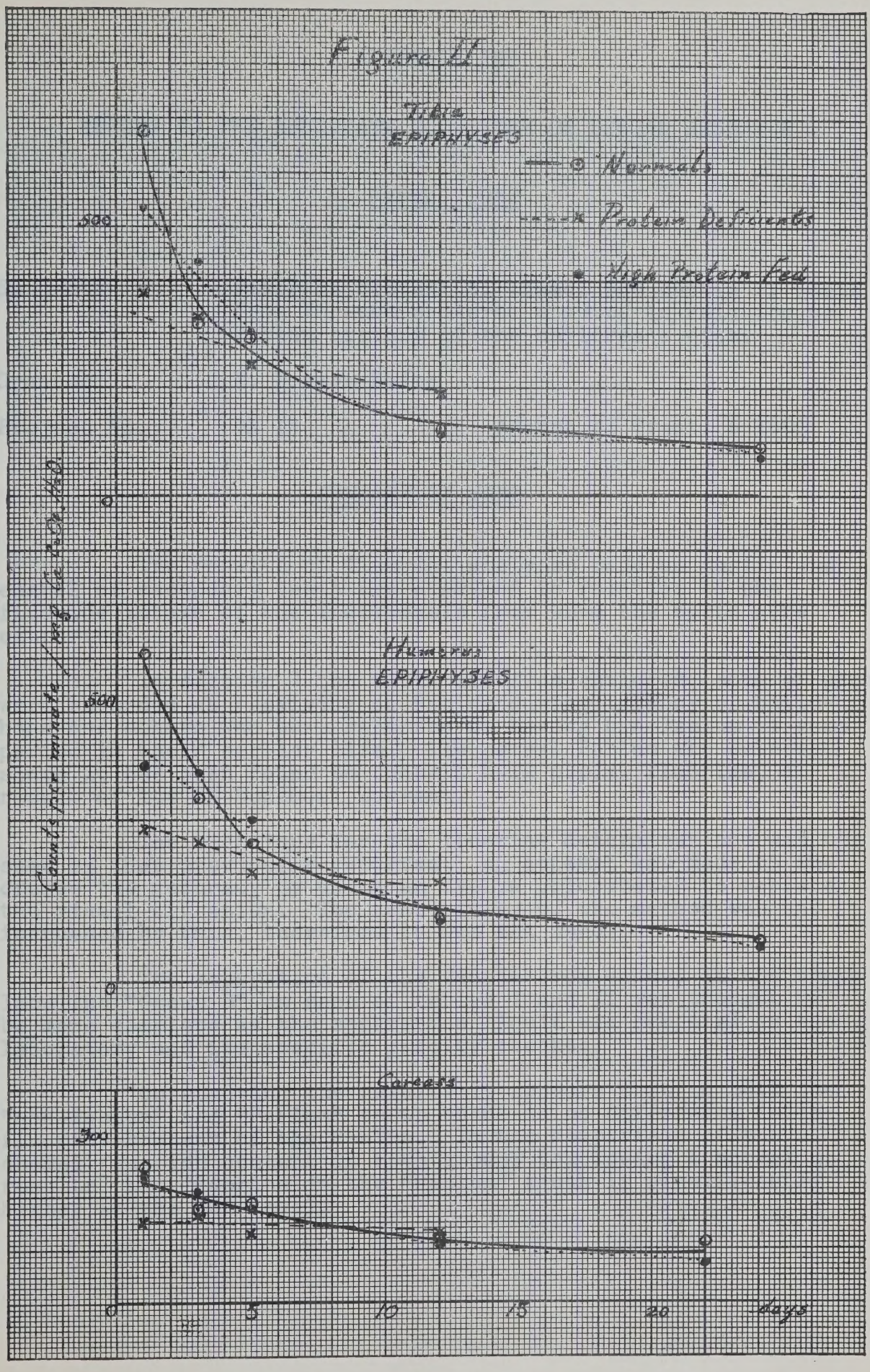
Figure 11
Tibia
EPIPHYSES
Normals
Protein Deficients
High Protein fed
500
0
Gm. ash per 100 gm. wet tissue
Humerus
EPIPHYSES
500
0
Carcass
300
0
5
10
15
20
days

APPENDIX "N"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: Fluoride content of enamel and dentin of teeth from Toronto children studied in relation to Pablum history of the children's diet.

Grantee: M. Doreen Smith

Project No.: DR 13 Amount of Grant: $ 1600

SUMMARY OF WORK

The results of fluorine analyses made on dentin and enamel of secondary teeth are examined in relation to the fluoride intake of the individuals from whom the teeth were extracted.

In section I the teeth are from children, some of whom were recorded as receiving no Pablum during infancy or childhood and others who were reported as having had Pablum at some period in their earlier life. The cereal Pablum was selected since it has been found to contain 6-12 p.p.m. fluorine, a result of its 2% bonemeal content.

The technique for the fluorine determinations was the same as that previously reported. An attempt was made to increase the accuracy by using an Evelyn colorimeter to replace the visual titration. The sensitivity of the method was not increased by this, therefore it was not used.

The separation of the dentin from enamel in the teeth labelled "Group B" on each of tables I and II was thought to be more complete as the separation liquid was prepared in the laboratory and its specific gracity checked.

On studying the results it appears that in both the "non-Pablum" and "Pablum" teeth there is no difference in the fluoride content of the dentin. In the comparison of the enamel fluoride content in both groups (excluding 4 anomalous results) the fluorine in the "Pablum" teeth is significantly higher at the 10% level.

Of the thirty "Pablum" teeth (excluding 2 high figures) only five ate Pablum after the age of four. Six of the thirty showed a higher percentage of fluorine in the enamel than any of the nineteen "non-Pablum" teeth (excluding the 2 high figures). Five of these had no caries and the sixth slight caries. The increase appears to be due to absorption of the fluorine.

In section II the results and averages of results of fluoride content of dentin and enamel of teeth from Stratford, Brantford and Sarnia are tabulated and a statistically significant difference found between both the dentin and enamel of Stratford teeth when compared with those from Sarnia. The results of the analyses of all the teeth studied are given in a bar graph. It is desirable to have more teeth analysed for inclusion in all the groups.

February 11, 1952

APPENDIX "N"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: Fluoride content of enamel and dentin of teeth from Toronto children studied in relation to Pablum history of the children's diet

Grantee: M. Doreen Smith

Project No.: DR 13 **Amount of Grant:** $1600

This report deals with the fluoride composition of dentin and enamel obtained from secondary teeth and viewed in relation to the fluoride intake of the individuals from whom the teeth were extracted.

The study is divided into two sections. In the first the report is given on the teeth from 2 groups of individuals. The members of one of these groups were recorded as having received no cereal in the form of Pablum at any time and the members of the second group according to the records received Pablum for a period during infancy and in some cases childhood.

In section two, the results are given for teeth collected from three districts of varying water composition with respect to fluorine.

Section I

The infant cereal Pablum has been found to contain from 6 to 12 p.p.m. fluorine. This is not surprising when the fact that bonemeal, a substance containing from 300 to 600 p.p.m. of fluorine forms 2% of its composition. Most of this fluorine is thought to exist in the form of fluoroapatite and the absorption of fluorine from bonemeal by humans has been questioned. Two reports (1,2) present evidence that from 36.8 - 53.6% of fluorine from bonemeal was absorbed by adult men (6 subjects). At the time this work was commenced and to-date no studies have been reported on infants or children with respect to fluorine absorption from bonemeal. In this connection it should be noted that bonemeal is being used to reinforce the flour being shipped to Newfoundland.

The purpose of the work undertaken here was to learn if any difference existed in the fluoride content of the enamel or dentin of teeth from children fed Pablum when compared with those from children receiving no Pablum. Thirty three teeth from Pablum-fed children and twenty one from non-Pablum fed children have been analysed. It has been very difficult to collect these teeth and unfortunately most of them showed severe caries.

Procedure

The first 24 of the Pablum teeth and the first 11 of the non-Pablum teeth were analysed by technician A and the remainder in each group by technician B. These are distinguished as groups A and B respectively in each table.

The separation of all teeth into dentin and enamel followed the Manley and Hodge procedure and the fluorine determinations were carried out by the method recommended in the Methods of Analysis of the Association of Official Agricultural Chemists and as used in previous reports by us.

Technician A cleaned the teeth and filed out the carious sections by hand. In following the separation procedure he used a British Drug House solution of bromoform containing 4% ethyl alcohol with a specific gravity about 2.65. When the ratios of his fluorine in dentin to fluorine in enamel were worked out, they were lower than those reported by McClure (3) or by Miss Harness (4). Incomplete separation of the enamel from the dentin would lower the ratios, this made it appear that the separation might be improved.

Technician B had the teeth cleaned and the carious sections drilled out through the courtesy of the Dental Faculty of the University of Toronto. She prepared the separation liquid from bromoform and acetone and when the specific gravity was checked at room temperature by a pyknometer it was found to be 2.71. After each separation the fractions were examined and if flecks of enamel could still be seen in the dentin, a second centrifuging step was introduced. As a check the separation technique was repeated on several dentin fractions and no additional enamel was obtained. It will be noticed that on the average higher ratios were obtained especially in the "Pablum" teeth analysed by technician B.

In all the work there is no question about the purity of the enamel and the teeth analysed by both technicians are considered together.

An in vitro experiment was carried out on each of two 1st molar teeth extracted from a 10 year old child. Each tooth was cut in half and the cut surfaces were covered with paraffin. One section of each tooth was placed in fluorine-free water and the other placed in a Pablum-water mixture. These tooth fractions were allowed to stand at room temperature for 16 days.

The Pablum-water mixture was renewed at the end of the first 8-day period. The tooth sections were then washed and brushed and finally put through the separation procedure. The enamel from each sample was analysed for fluorine.

An attempt was made to increase the sensitivity of the chemical method of determining fluorine by use of the Evelyn colorimeter instead of the visual comparison in Nessler tubes at the titration stage of the determination. This met with some success but gave no increase in accuracy and was not introduced into the method.

Results and Discussion

In tables I, II and III the results of analyses of the "Pablum" and "non-Pablum" teeth are given, and in table IV the results of the in vitro experiment with teeth and Pablum are shown.

Before commenting on these it should be noted that the greater number of the teeth had caries and that the sampling of the "non-Pablum" teeth was especially poor since the last 7 teeth in this group were from one patient. Unfortunately this was not noticed until after the analyses were made. The age range of the "Pablum" teeth is 7 to 15 and that for the "non-Pablum" 6 to 17. Even in these young permanent teeth the general tendency for an increase in fluorine with age may be seen. The sampling in both groups takes care of the age range fairly well.

In the "non-Pablum" teeth the results for teeth numbers 6 and 10 appeared to be anomalous therefore when statistical comparisons were made with the "Pablum" teeth, they were made both with and without these two teeth.

In the "Pablum" groups of teeth numbers 10 and 33 were unusually high and comparisons of the "Pablum" teeth also were made including and excluding these results.

On examination of the dentin averages there is no apparent difference, although in groups A of tables I and II it is suspected that in some cases there might be some contamination with enamel.

In table III when the results for the enamel of all the teeth in the "non-Pablum" group are compared statistically with all of those in the "Pablum" group there is no significance at the 10% level. When the four teeth with anomalous or high results are left out the enamel for the "Pablum" teeth is higher than that for the "non-Pablum" when compared at the 10% level. It has to be kept in mind that the intake of Pablum by the patients from whom these teeth were removed may have been in some cases, intermittant, and only for a short time, especially as this cereal some years ago was more expensive than other cereals.

In the nineteen "non-Pablum" teeth (excluding the two high figures) none showed a higher fluorine content than 0.0088% whereas in the thirty teeth of the "Pablum" group (excluding the two high figures) six were higher than this having a range of 0.0099% to 0.015%. One of the six ate Pablum up to the age of 10 years, the others ate it for from 1 to 4 years according to our records. Five of these six teeth showed no caries and the sixth very slight. The dentin/enamel ratio in these six teeth was close to one. This, no doubt would be due to no corresponding increase in the fluorine of the dentin with that of the enamel. Teeth from high fluoride water areas show increase in fluorine content of both dentin and enamel. However, the fluorine in water is in a soluble form and not in the fluorapatite form which would be the case with Pablum.

The indication that fluorine from Pablum may increase the fluoride content of the enamel of teeth is interesting because if so it cannot be a topical effect since Pablum was not consumed after the eruption of these teeth.

In our laboratory we now have evidence of absorption and retention of fluorine from Pablum by young infants. This has been obtained by a series of balance studies carried out on five infants. Therefore, it is not surprising that if a reasonable amount of Pablum is consumed over a period of time, it could affect the fluoride content of the teeth.

It is hoped that more analyses can be made to add to each group and it also appears to be desirable to analyse whole teeth in each group.

In table IV the <u>in vitro</u> experiment showed the enamel appeared to absorb or adsorb fluorine from the Pablum.

<u>Section II</u>

Teeth received from Stratford, Brantford and Sarnia were separated into dentin and enamel and analysed for fluorine by the same procedure as those in section I. Analyses on the Stratford and Sarnia teeth were carried out by technician A.

The results from the analyses are shown in tables VI, VII and the averages of the results in table VIII.

Statistical comparisons of the analyses for the dentin of the Stratford teeth was made with that of the dentin for the Sarnia teeth and the difference was significant at the 1% level. A similar comparison of the enamel in both groups showed the difference also to be significant, at the 1% level.

The numbers analysed are small but the trends seem fairly definitely towards an increased content of fluorine in both the dentin and enamel of the Stratford over the Sarnia teeth. The ratios of the enamel to dentin in both groups is similar. This also shows that the increase in fluoride content is at about the same rate in the dentin as the enamel of the Stratford teeth. Most of the Sarnia teeth showed severe caries. The accompanying bar graph shows the Brantford teeth tend to fill a place between the Stratford and Sarnia teeth with respect to fluoride content.

It is hoped more teeth can be analysed in each of these groups.

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Table I - Non-Pablum Teeth (Group A)

No.	Age	Sex	Type of Tooth	Degree of Caries	% Fluorine in Dentin	% Fluorine in Enamel	D/E
1	6		1st molar	slight	0.0095	0.0024	4.0
2	6		1st molar	slight	0.0078	0.0027	2.9
3	6		1st molar	slight	0.0080	0.0030	2.6
4	8		1st molar	severe	0.0065	0.0066	1.0
5	9		1st molar	severe	0.0088	0.0088	1.0
6	9		1st molar	moderate	0.0085	0.026(5)	0.3
7	10	F	2nd bicuspid	none	0.010(6)	0.0086	1.2
8	10	F	2nd bicuspid	none	0.0097	0.0055	1.8
9	15	F	1st incisor		0.013(3)	0.0046	2.9
10	17	M	U. 1st molar	severe	0.011(2)	0.023(6)	0.5
11	17	M	U. 1st bicuspid	severe	0.012(6)	0.005(1)	2.4

Table I - Non-Pablum Teeth continued (GroupB)

12	13	F	L.R. 1st molar	severe	0.020(4)	0.0071	2.9
13	13	F	U.L. 1st molar	severe	0.023(9)	0.0058	4.0
14	13	F	U.R. 1st molar	severe	0.030(4)	0.0026	11.0
15#	14	F	U.L.2nd bicuspid	severe	0.016(8)	0.0052	3.1
16	14	F	L.R.1st bicuspid	severe	0.014(6)	0.0039	3.7
17	14	F	U.L.1st bicuspid	slight	0.019(3)	0.0048	4.0
18	14	F	L.L. 1st molar	severe	0.014(9)	0.0030	4.9
19	14	F	U.L. 1st molar	very severe	0.021(4)	0.0023	9.3
20	14	F	U.R.1st bicuspid	severe	0.010(6)	0.0000	
21	14	F	U.R.2nd bicuspid	severe	0.012(2)	0.0000	

Numbers 15-21 from same patient.

Table I - Non-fashion teeth (Group A)

No.	Age	Sex	Type of tooth	Degree of Caries	β Fluorine in Dentin	β Fluorine in Enamel	%
1	6		1st molar	slight	0.0095	0.0024	6.0
2	9		1st molar	slight	0.0078	0.0027	5.9
3	8		1st molar	slight	0.0080	0.0030	5.6
4	8		1st molar	severe	0.0543	0.0046	1.0
5	9		1st molar	severe	0.0088	0.0088	1.0
6	9		1st molar	moderate	0.0085	0.0246(5)	0.3
7	10	F	2nd bicuspid	none	0.0170(6)	0.0086	1.2
8	10	M	2nd bicuspid	none	0.0097	0.0055	1.8
9	15	F	1st incisor		0.0243(5)	0.0046	5.3
10	17	M	U. 1st molar	severe	0.0211(2)	0.0234(6)	0.5
11	17	M	U. 1st bicuspid	severe	0.0126(6)	0.0053(4)	2.4

Table I - Non-fashion teeth continued (Group A)

12	13	F	L.m. 1st molar	severe	0.0204(4)	0.0071	2.9
13	13	F	U.L. 1st molar	severe	0.0243(9)	0.0058	4.0
14	13	F	U.R. 1st molar	severe	0.0240(4)	0.0026	12.0
15*	14	F	U.L.2nd bicuspid	severe	0.0146(8)	0.0052	3.1
16	14	F	L.R.1st bicuspid	severe	0.0144(6)	0.0039	3.7
17	14	F	U.L.1st bicuspid	slight	0.0219(3)	0.0048	4.0
18	14	F	L.L. 1st molar	severe	0.0214(9)	0.0030	6.9
19	14	F	U.L. 1st molar	very severe	0.0211(4)	0.0028	9.3
20	14	F	U.R.1st bicuspid	severe	0.0140(6)	0.0000	
21	14	F	U.R.2nd bicuspid	severe	0.0112(5)	0.0000	

* Numbers 15-21 from same patient.

Table II - Pablum Teeth (Group A)

No.	Age	Sex	Type of Tooth	Degree of Caries	% Fluorine in Dentin	% Fluorine in Enamel	D/E
1	7		1st molar	severe	0.0062	0.0059	1.1
2	7		1st molar	severe	0.0080	0.0037	2.1
3	8		1st molar	moderate	0.0057	0.0051	1.1
4	8		1st molar	severe	0.0064	0.0035	1.8
5	8		1st molar	severe	0.0068	0.0068	1.0
6	8		1st molar	severe	0.0073	0.0057	1.3
7	10		L.L. 2nd molar		0.0066	0.0066	1.0
8	10		1st molar	severe	0.010(6)	0.0060	1.8
9	10	M	L. 1st molar	severe	0.0059	0.0031	1.9
10	10	M	U. 1st molar	severe	0.052(0)	0.074(9)	0.7
11	10	M	L. 1st molar	severe	0.0062	0.0041	1.5
12	11	M	U. 2nd molar	severe	0.0077	0.0045	1.7
13	11	F	L.R. 1st molar		0.0088	0.0099	0.9
14	12	F	U.R. 1st molar		0.010(2)	0.012(6)	0.8
15#	12	M	1st bicuspid	none	0.013(5)		
16#	12	M	1st bicuspid	none	0.012(0)	0.011(0)	1.1
17#	12	M	incisor	none	0.0046	0.0042	1.1
18#	12	M	incisor	none	0.0060	0.0034	1.7
19	13	F	L.R. molar	severe	0.0062	0.0030	2.0
20#	13	F	1st bicuspid	none	0.010(8)	0.0060	1.8
21#	13	F	1st bicuspid	none	0.0080	0.0040	2.0
22	14		bicuspid		0.010(1)	0.0051	2.0
23	15	F	L. 1st molar	severe	0.0052	0.0034	1.5
24	15	F	U.R. 1st molar		0.019(4)	0.011(3)	1.7

- ate Pablum up to the age of 10 to 12 years

Table II - Fabian Tests (Group A)

No.	Age	Sex	Type of Teeth	Degree of Caries	% Fluorine in Smalts	% Fluorine in Enamel	% F
1	7		1st molar	severe	0.0062	0.0059	1.7
2	7		1st molar	severe	0.0080	0.0057	0.1
3	8		1st molar	moderate	0.0057	0.0051	1.1
4	8		1st molar	severe	0.0064	0.0059	1.8
5	8		1st molar	severe	0.0050	0.0058	1.0
6	8		1st molar	severe	0.0075	0.0057	1.3
7	10		L.L. 2nd molar		0.0056	0.0056	1.0
8	10		1st molar	severe	0.010(6)	0.0060	1.8
9	10	M	L. 1st molar	severe	0.0059	0.0051	1.9
10	10	M	U. 1st molar	severe	0.052(0)	0.074(9)	0.7
11	10	M	L. 1st molar	severe	0.0062	0.0063	1.5
12	11	M	U. 2nd molar	severe	0.0077	0.0045	1.7
13	11	F	L.R. 1st molar		0.0058	0.0059	0.5
14	12	F	U.R. 1st molar		0.010(2)	0.012(5)	0.8
15½	12	M	1st bicuspid	none	0.013(5)		
16½	12	M	1st bicuspid	none	0.012(0)	0.011(0)	1.1
17½	12	M	incisor	none	0.0046	0.0042	1.1
18½	12	M	incisor	none	0.0060	0.0034	1.7
19	13	F	L.R. molar	severe	0.0062	0.0050	2.0
20½	13	F	1st bicuspid	none	0.010(8)	0.0060	1.8
21½	13	F	1st bicuspid	none	0.0050	0.0040	2.0
22	14		bicuspid		0.010(1)	0.0051	2.0
23	15	F	L. 1st molar	severe	0.0052	0.0034	1.5
24	15	F	U.R. 1st molar		0.013(4)	0.011(3)	1.7

½ - are Fabian up to the age of 10 to 12 years

Table II - Pablum Teeth continued (Group B) - (Fed Pablum 1 to 4 years).

No.	Age	Sex	Type of Tooth	Degree of Caries	% Fluorine in Dentin	% Fluorine in Enamel	D/E
25	10	F	L.L. 2nd bicuspid	very slight	0.011(0)	0.0095	1.2
26	10	F	L.R. 1st molar	severe	0.011(2)	0.0029	3.8
27	10	F	L.L. 1st molar	severe	0.016(1)	0.0015	10.6
28	11	M	L.R. 1st molar	severe	0.013(9)	0.0059	2.3
29	11	M	L.L. 1st molar	severe	0.012(9)	0.0046	2.8
30	14	M	U.R. 1st molar	severe	0.018(5)	0.0063	2.9
31	15	F	U.L. cuspid	none	0.010(9)	0.015(1)	0.73
32	15	F	U.R. cuspid	none	0.013(7)	0.0065	2.0
33	14	M	U.L. 1st molar	severe	0.088(9)	0.029(4)	3.0

Note: Table III is on the following page.

Table IV

% Fluoride in Enamel

Tooth No.	Tooth in Fluoride Free Water	Tooth in Pablum Water Mixture
1	0.0029	0.0057
2	0.0022	0.0039

Table IV

Tooth No.	Tooth in Fluoride Free Water	Tooth in Fabian Water Mixture
1	0.0029	0.0057
2	0.0022	0.0039

Table III

Averages of % Fluorine			Group Comparisons of Enamel	
	Dentin	Enamel	Calculated "t"	Tabulated "t" at 10% level
Total "non-Pablum"	0.0133	0.0064	.823	1.68 (no significance)
Total "Pablum"	0.0134	0.0089		
"non-Pablum" minus 2 irregular results	0.036	0.0044	1.87	1.68
"Pablum" minus 2 irregular results	0.100	0.0061		

Table V - Fluorine Content of Sarnia Teeth

No.	Age	Sex	Type of Tooth	Degree of Caries	% Fluorine in Dentin	% Fluorine in Enamel	D/E
1	8	F	U.R. 1st molar	moderate	0.0063	0.0073	0.8
2	8	F	L.L. 1st molar	severe	0.0069	0.0070	1.0
3	9	F	L.R. 1st molar	severe	0.0089	0.0067	1.3
4	11	M	L.L. 1st molar	severe	0.0068	0.0039	1.7
5	12	M	R. 1st molar	severe	0.010(7)		
6	12	F	1st molar	severe	0.0078	0.0032	2.4
7	15	F	L.L. molar	severe	0.029(5)	0.017(8)	1.6
8	16	M	L.R. bicuspid	severe	0.0081	0.0087	1.0
9	16	M	1st molar	severe	0.013(0)		
10	25	M	M.U. cuspid	moderate	0.014(5)	0.0031	4.6
				Average =	0.011(2)	0.0072	1.8

Table V - Fluorine content of Garnia teeth

No.	Age	Sex	Type of tooth	Degree of Caries	% Fluorine in Dentin	% Fluorine in Enamel	D/E
1	8	F	U.R. 1st molar	moderate	0.0069	0.0079	0.8
2	8	F	L.L. 1st molar	severe	0.0069	0.0070	1.0
3	9	F	L.R. 1st molar	severe	0.0089	0.0067	1.3
4	11	M	L.L. 1st molar	severe	0.0068	0.0039	1.7
5	12	M	R. 1st molar	severe	0.010(1)		
6	12	F	1st molar	severe	0.0078	0.0032	2.4
7	15	F	L.L. molar	severe	0.029(5)	0.017(8)	1.6
8	16	M	L.R. bicuspid	severe	0.0081	0.0087	1.0
9	16	M	1st molar	severe	0.015(0)		
10	25	M	R.U. cuspid	moderate	0.014(5)	0.0031	4.6
				Average =	0.011(2)	0.0072	1.8

Table VI-Fluorine Content of Stratford Teeth

No.	Age	Sex	Type of Tooth	Degree of Caries	% Fluorine in Dentin	% Fluorine in Enamel	D/E
11	14	F	incisor	none	0.030(6)	0.018(9)	1.6
12	17	M	molar		0.027(9)	0.014(0)	1.9
13	17	F	molar	moderate	0.047(8)	0.020(0)	2.3
14	21	M	molar		0.046(7)	0.022(3)	2.1
15	25	F	bicuspid	severe	0.075(5)	0.039(3)	1.9
				Average =	0.045(7)	0.022(9)	2.0

Table VII-Fluorine Content of Brantford Teeth

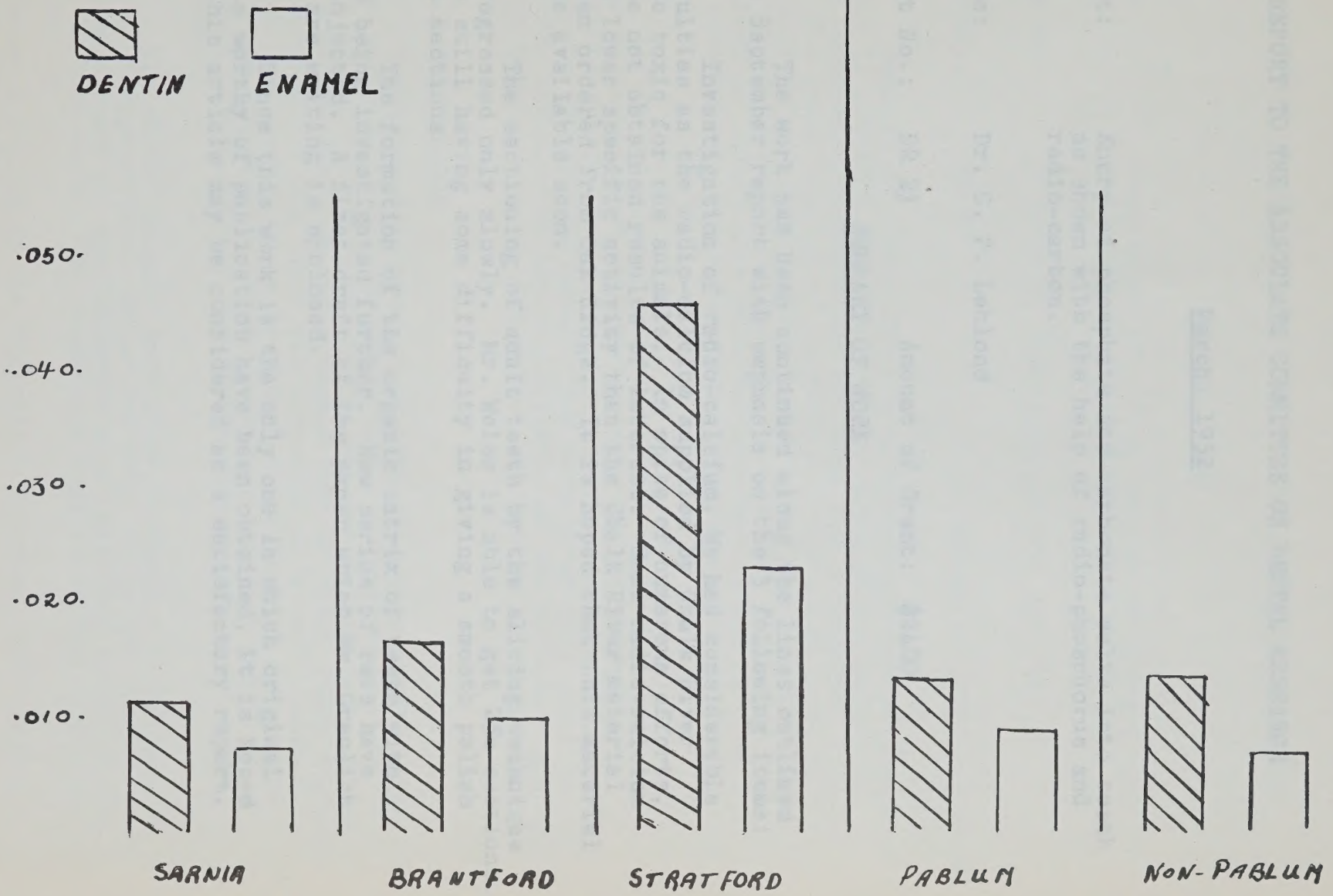
16	8	F	U.R. 1st molar	severe	0.019(3)	0.017(9)	1.1
17	8	M	2nd molar	severe	0.0086	0.0097	0.9
Average including previously reported results					0.0167	0.0098	

Table VIII- Comparison of Average Fluoride Content of Stratford, Brantford and Sarnia Teeth

	No. of Teeth	Average % Fluoride in Dentin	Average % Fluoride in Enamel	D/E	
Stratford	5	(0.027-0.075) 0.046	(0.014-0.039) 0.023	2.0	(1.6-2.3)
Brantford #	11	(0.0037-0.032) 0.017	(0.009-0.035) 0.010	2.4	(2.1)* (0.9-5.2)
Sarnia	10	(0.006-0.030) 0.012	(0.003-0.018) 0.007	1.8	(0.8-4.6)

Including previously reported results.
* If one high-ratio tooth is omitted.

% FLUORIDE IN DENTIN AND ENAMEL



% FLUORIDE IN DENTIN
ENAMEL AND
DENTIN ENAMEL
.010
.020
.030
.040
.050
SMOKING
GRADE 3 BRACHYCEPHALIC
GRADE 3 DOLICHOCEPHALIC
NATIVE BORN
NON-NATIVE BORN

APPENDIX "O"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: Entry of phosphate and carbonate salts into teeth as shown with the help of radio-phosphorus and radio-carbon.

Grantee: Dr. C. P. Leblond

Project No.: DR 23 Amount of Grant: $2400

SUMMARY OF WORK

The work has been continued along the lines outlined in the September report with emphasis on the 3 following items:

1) Investigation of radio-calcium. We had considerable difficulties as the radio-calcium supplied by Chalk River was too toxic for the animals. In spite of numerous efforts, we have not obtained results of interest. Some radio-calcium with a lower specific activity than the Chalk River material has been ordered from Oak Ridge. It is hoped that this material will be available soon.

2) The sectioning of adult teeth by the slicing technique has progressed only slowly. Mr. Weiss is able to get 25u sections. We are still having some difficulty in giving a smooth polish to the sections.

3) The formation of the organic matrix of teeth with C^{14} is being investigated further. New series of rats have been injected. A first draft of the paper which Mr. Greulich and I are writing is enclosed.

Since this work is the only one in which original results worthy of publication have been obtained, it is hoped that this article may be considered as a satisfactory report.

APPENDIX "O"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Title:- Deposition of C^{14} in incisor of newborn rats following administration of $NaHC^{14}O_3$

Article by:- Richard C. Greulich[1] and C.P. Leblond

Project No.: DR 23 Amount of Grant: $ 2400

Studies of teeth with the radio-autographic technique have made it possible to visualize the sites of deposition of labeled phosphate (Bélanger and Leblond, '50 ; Bélanger, '51). Since tooth salts consist of phosphatases and carbonates, it was of interest to know whether carbonates would have the same pattern of localization as did the phosphates. Therefore, it was decided to inject rats with radio-carbon in the form of sodium bicarbonate, and to prepare radio-autographs of their teeth.

After this experiment was begun, it became apparent that the histological technique employed in the preparation of the radio-autographs could cause dissolution of the inorganic constituents of bone and teeth. The purpose of this paper is to describe and discuss the significance of the radio-autographic reactions obtained in the absence of the inorganic constituents of teeth.

Materials and Methods

Since the biological half life of inorganic C^{14} salts had been shown to be of very short duration in adult animals (Skipper et al. '49; Govaerts, '50), newborn animals were chosen for this experiment. It was presumed that in these animals growth and development of the teeth was proceding at a rapid rate, and hence there would be an increased likelihood of C^{14}-bicarbonate being utilized in these processes. Hence a total of ten newborn albino rats of the Fisher strain were used in the experiment.

Six of the animals were given a single subcutaneous injection of approximately 20 μc $NaHC^{14}O_3$, while the remaining four animals received approximately 40 μc $NaHC^{14}O_3$ by the same route. In both cases, the injections were administered within

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[1] Predoctoral Fellow in the Biological Sciences, U.S. Atomic Energy Commission

twelve hours after birth. The animals were sacrificed by means of chloroform at three time intervals after injection, namely, four hours (2 animals given 20 μc and 1 given 40 μc).

Whole mandibles were fixed in a mixture of absolute alcohol and 40% neutral formalin in a ratio of 3:1. These tissues were then dehydrated in dioxane and embedded in paraffin without prior decalcification. Sections were cut at 5 μc for histological examination and for radio-autography.

Sections for histological examination were treated in the following manners. Some sections were stained routinely with hematoxylin-eosin, and of these some were subsequently treated by the von Kossa technique. Other sections were treated directly by the von Kossa technique, and still others were deparaffinized and examined unstained in polarized light.

Half of the sections for radio-autography were treated with saliva for 10 minutes at 60°C to remove glycogen, and then, along with untreated sections, were stained by hematoxylin-eosin. These preparations were then radio-autographed by the "coating technique" (Leblond et al. '48; Gross et al, '50) utilizing the rather slow but fine-grained Eastman Kodak NTB_3 emulsion. (This emulsion was obtained in pellicles which had to be soaked in a 1% aqueous solution of Duponol for 24 hours prior to melting).

The coated radio-autographs were left to expose in the dark at 0°C for periods ranging from 8 to 10 months. Following exposure the slides were subjected to routine photographic development and fixation, dehydration in xylene, and mounting under Canada balsam.

Results

The results of the tests on preparations treated by the von Kossa technique and on those examined in polarized light can be summarized briefly as follows. Teeth stained with hematoxylin-eosin and treated by the von Kossa technique did not exhibit areas of brownish-black precipitation indicative of the presence of phosphates.

A description of radio-autographs will be limited for the most part to those obtained from incisor teeth, for although molar radio-autographs generally exhibited the same type of reactions as did incisors, some differences existed. As these differences may represent variations in molar formation compared to incisor formation, further experiments are now underway to determine their extent and significance.

Radio-autographic reactions in incisors may for convenience be classified on a basis of their locale and intensity into three types.

The first and most striking type of radio-autographic reaction was seen in the "dentinogenic" system, which for the sake of simplicity is taken to include the odontoblastic epithelium, the predentin, and the dentin proper. At four hours a precisely localized and relatively intense radio-autographic reaction occurred over the region of predentin immediately adjacent to the odontoblastic epithelium (figs. 1 and 2, PD). It was noted that, even at this early interval, no reaction was present over the odontoblastic epithelium itself. Moreover, no reaction was observed over the dentin present (fig. 2, D). The latter observation was further emphasized by the fact that in the area where no dentin but only predentin was present, there was a reaction, as illustrated in figure 1. This predentinal reaction extended throughout the circumference of the incisor, but was seen to be most marked on that side of the incisor upon which amelogenesis was to occur. By twenty-four hours, little change in the intensity of this reaction was observed, but its distribution was slightly altered. In some of the areas where only predentine was present at the early time interval some dentin was now found to be present, and that portion of it most nearly in apposition with the predentin was seen to exhibit a radio-autographic reaction. Reaction of the full width of the predentinal region was also evident at this interval. In contrast to the reactivity of predentin at the earlier intervals, at seventy-two hours this material was almost totally unreactive, while much reactivity was seen to lie over the dentin proper, in most areas extending to the dentino-enamel junction (fig. 3). No odontoblastic reaction was seen at this or any other time interval.

The second, and somewhat less striking type of radio-autographic reaction was seen in the "amelogenic" system, which, again for the sake of simplicity, is taken to include the ameloblastic epithelium (inner epithelium of the enamel organ), the pre-enamel, and the enamel proper. Although the pattern of the "amelogenic" reaction is in most respects similar to that of the "dentinogenic" reaction, there are some points of difference. At four hours, a line of radio-autographic reaction was observed at the tips of the ameloblasts (figs. 1 and 2, PE). This reaction seemed to lie over the pre-enamel in close apposition to the ameloblastic layer, and was observed to be present only on that side of the incisor upon which active amelogenesis was to occur. In contrast to the lack of radio-autographic reaction of the odontoblastic epithelium the ameloblastic epithelium exhibited a light but definite reaction at this time, and this reaction was maintained throughout the 72-hour period. In the preparations

illustrated in figures 1 and 2, little if any definitive enamel was present. By twenty-four hours this line of reaction was displayed by the rather thin layer of enamel which had been laid down at that time. The pre-enamel showed a reduced intensity of reaction, and by seventy-two hours, the reaction line was confined almost entirely to the enamel layer, being most intense in the region of the dentino-enamel junction (fig. 3). However, a light radio-autographic reaction similar in intensity to that displayed by the ameloblastic epithelium was still observed in the pre-enamel layer.

The third type of radio-autographic reaction was that exhibited by structures other than those mentioned above which are also concerned in the development of teeth. These include dental pulp, and those constituents of the enamel organ not dealt with heretofore. In general, at all time intervals, the dental pulp and the stellate reticulum of the enamel organ exhibited definite but completely homogeneous radio-autographic reactions which could not be related to particular structural components of these tissues. These reactions, however, were seen to diminish in intensity with time, relative to the reactions discussed above. Of particular interest was the fact that the region of the stratum intermedium of the enamel organ displayed a definite radio-autographic reaction (fig. 1) which was maintained in intensity throughout the seventy-two hour period.

Incisors from animals which received 40 μc $NaHC^{14}O_3$ showed no differences in the sites of C^{14} retention, and in fact, showed only slight increases in intensity of reaction when these teeth were compared with those from animals receiving only half the dose of bicarbonate.

It should also be emphasized that the removal of glycogen by salivary digestion had no noticeable effects upon the sites of C^{14} retention or upon the intensity of radio-autographic reaction.

Discussion

Previous observations[1] on bone indicated that preparations fixed 24 hours in Carnoy fluid and routinely stained with hematoxylin-eosin no longer contained appreciable quantities of inorganic salts. Since Carnoy fluid is relatively acid in nature (pH 3.5), it was not surprising that bone salts were removed by it. For that reason, a neutral fixative was chosen in this experiment. However, there still remained the question whether the acidity of Harris' hematoxylin (pH 3.%) was in itself sufficient to remove bone salts from sections. Routine staining with hematoxylin in this Laboratory requires immersion of the

slide in the stain for five minutes or longer depending upon the age of the stain, and further, differentiation accomplished by short treatment in acid alcohol. Hence, the von Kossa technique was utilized to establish the presence of phosphates in the areas of amelogenesis and dentinogenesis of hematoxylin-eosin preparations. Since no brownish-black precipitation indicative of the presence of phosphates occurred in these areas, it is assumed that, even following fixation in a neutral medium, the acidity of the hematoxylin solution used for staining was sufficient to remove the inorganic constituents of the teeth That these salts were not removed prior to staining was evidenced both by the appearance of a positive von Kossa reaction in unstained preparations and also by birefringence of areas of amelogenesis and dentinogenesis in unstained preparations examined in polarized light. These results indicate, then, that any autographic reactions exhibited by areas of dentine and enamel can be ascribed to the organic components of these structures.

Thus the striking radio-autographic reaction of the predentinal region at four hours (fig. 1) seems to indicate a rapid incorporation of the C^{14}- bicarbonate ion into one or more of the organic components of this material. It should be borne in mind that not only were inorganic constituents of teeth removed by the histological procedure, but also treatment of these preparations in fat solvents such as xylene, dioxane, etc., probably removed substances of a lipoidal nature. Moreover, water soluble substances such as monosaccharides, amino acids, urea and some proteins would be extracted during fixation and staining. The substances retained in histological sections may then be classified as proteins, carbohydrates, or carbohydrate-protein complexes. There may also be other substances such as, for instance, lipo-proteins, but they are believed to be too small in amount to play a significant role in the radio-autographic reactions observed. It is therefore concluded that the radioactive carbon in the form of bicarbonate was utilized in the metabolism of tissues to produce within four hours substances of all or some of the three types listed.

That the synthesis of this organic matrix must occur before subsequent deposition of tooth salts is borne out by these results. Moreover, it appears that the relative rate at which this material is elaborated plays a fundamental role in determining the definitive shape of the tooth. Evidence for this assumption is presented by the fact that the predentinal reaction at four hours is most intense on that side of the incisor upon which enamel will be laid down. Perhaps the curvature of the rat incisor could be explained on such a basis. If one assumes that the cellular elements concerned with the continual apposition of dentin and enamel in post-natal life constitute a rather rigid and unyielding framework in which the incisor lies, then the fact that more dentin is deposited on the labial aspect than on the lingual aspect would probably cause the normal

curvature. Of course, the fact that enamel, too, is being deposited on the labial but not the lingual aspect of the tooth would further accentuate the curvature. Such an explanation is undoubtedly far too simplified, but gives a basis upon which further experiments may be built.

An additional problem concerning the fate of dentin is raised when comparison is made between figures 2 and 3. These are microphotographs of similar areas of the incisor at 4 and 72 hours after injection of C^{14}-bicarbonate, respectively. It can be seen that at 4 hours, some dentin is present which does not exhibit a radio-autographic reaction (fig. 2). By seventy-two hours, however, not only does the dentin exhibit a radio-autographic reaction (due presumably to the same area of organic matrix seen in the predentin at four hours which has moved outward), but also that reaction extends right up to the dentino-enamel junction (fig.3). Thus arises the question, what was the fate of the non-reactive dentin seen at four hours? At least three alternative answers can be considered in answering this question. First, it is possible that some degree of reabsorption of dentinal material may take place at the dentino-enamel junction. Second, there could possibly be a "diffusion" of the reactive predentinal material seen at four hours into the non-reactive dentin, so that by seventy-two hours the reaction would be homogeneous. This could be accomplished via the "dental lymph" of the dentinal tubules. Third, there could possibly be an "attenuation" of the most superficial layers of dentin during growth, so that by seventy-two hours the relatively thick layer of unreactive dentin seen at four hours would be reduced in thickness to a point at which it would be completely obscured by the radio-autographic reaction immediately adjacent to it. Certainly, this phenomenon, if it is real and not imagined, would also play an important role in determining the ultimate shape of the tooth. Further experiments are now being undertaken to investigate this problem.

Summary

The radio-autographic technique has been employed to assess the extent and site of C^{14} deposition in incisors of albino rats sacrificed at 4, 24 and 72 hours after the administration of a single subcutaneous injection of $NaHO^{14}O_3$. Radio-autographic reactions were seen at all time intervals. A reactive band was visible in the predentin at 4 hours, at the limit between predentin and dentin at 24 hours, and in the dentin at 72 hours. Lesser reactions were observed in the pre-enamel and enamel. Investigation by means of the von Kossa technique revealed that the inorganic constituents of the teeth had been removed in the histological procedure and, therefore, the radio-autographic reactions observed were those of the organic matrix of enamel and dentin.

It is concluded that the dentin matrix is formed as predentin and moves outward to transform into dentin itself. A similar mechanism may lead to the formation of enamel.

ACKNOWLEDGMENT

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PLATE 1

EXPLANATION OF FIGURES

All figures are radio-autographs of portions of rat incisor stained with hematoxylin-eosin.

SI: stratum intermedium; A: ameloblastic epithelium; PE: pre-enamel; E: enamel; D: dentin; PD: predentin; O: odontoblastic epithelium; DP: dental pulp.

1 Portion of rat incisor taken from animal injected with approximately 40 μc $NaHC^{14}O_3$ and sacrificed four hours after injection. x 135.

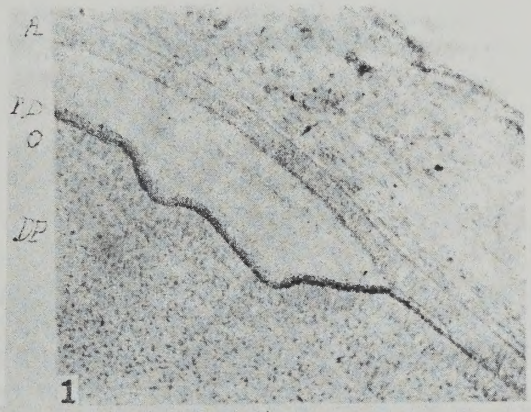
An intense radio-autographic reaction is seen to lie over the predentin immediately adjacent to the odontoblast. The split which occurred between the the predentin and pre-enamel serves to emphasize the preciseness of the reaction. A less intense line of reaction is seen to lie over the thin pre-enamel layer adjacent to the ameloblasts. The ameloblasts themselves exhibit a diffuse reaction, as does the dental pulp. The cells of the stratum intermedium are clearly demarcated by the line of reaction overlying them. The odontoblasts are unreactive.

2 Portion of rat incisor taken from animal injected with approximately 20 μc $NaHC^{14}O_3$ and sacrificed four hours after injection. x 226.

This area is somewhat closer to the incisal border than that illustrated in figure 1, and is seen to contain a layer of dentin. The radio-autographic reactions of the predentin and pre-enamel stand out clearly, but the dentin is unreactive. The reaction of the ameloblasts is diffuse.

3 Portion of rat incisor taken from animal injected with approximately 40 μc $NaHC^{14}O_3$ and sacrificed seventy-two hours after injection. x 245.

This area is similar to that illustrated in figure 2, and shows that the radio-autographic reaction of dentin extends close to the dentino-enamel junction. No layer of unreactive dentin comparable to that in figure 2 is seen. Reaction of enamel also extends to the dentino-enamel junction.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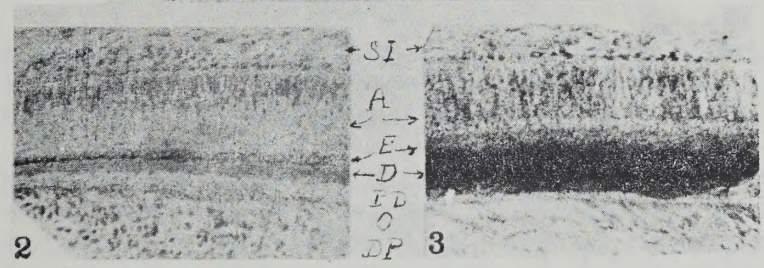
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APPENDIX "P"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: Oral Pathology and Bacteriology

Grantee: Dr. Gerald Shklar

Project: Fellowship Grant

Amount of Grant: $1500

Dr. Gerald Shklar continues to be a very hard working, well oriented student. He is obviously the best of six graduate students in this department. His interests are broad. His working capacity excellent.

He presented a seminar on, "Allergic Phenomena and Gingival Disease" to the faculty which was well organized and well received. His major interest at present is in the subject of "stress" and the periodontal tissues. Sections have been prepared of some of his experimental animals and he is at present making preliminary microscopic observations on this material. He is conducting several experiments attempting to repeat some of the work of Selye with particular interest in the oral tissues.

30 January, 1952 Irving Glickman

APPENDIX "Q"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

PAPERS PUBLISHED

Committee Paper No.	Title and Author	Reference
1	New aspects of periodontal research, by H.K. BOX	Presented at National Convention of Canadian Dentists, Toronto, 29 May, 1946. (App. "C", 5th Mtg. A.C.D.R., 25 Feb., 1947).
2	Concerning the pathogenesis of periodontal disease, by H.K. BOX	J. Periodontology, 19: 11-20. 1948. (App. "G", 7th Mtg. A.C.D.R., 2 Mar., 1948).
3	The effect of various inorganic salts on the solubility of calcium phosphate, tooth enamel and whole teeth in lactic acid, by J.J. RAE and C.T. CLEGG	J. Dent. Res. 27: 52-53. 1948.
4	Changes in the calcium and phosphate concentrations of saliva and inorganic salt solutions on shaking with calcium phosphate, by J.J. RAE and C.T. CLEGG	J. Dent. Res. 27: 54-57. 1948.
5	An improved method for processing acrylic dentures, by D.R. CHRISTIE	J. Can. Dent. Assoc., 14: 303-317. 1948. (App. "R", 8th Mtg., A.C.D.R., 26 Oct., 1948).
6	The therapeutic properties of periodontal cement packs, by W.J. LINGHORNE and D.C. O'CONNELL	J. Can. Dent. Assoc., 15: 199-205. 1949.
7	Phosphatase in saliva, by J.T. DENTAY and J.J. RAE	J. Dent. Res., Feb. 1949. 4 pp.
8	The treatment of acute oral Vincent's infection, by W.J. LINGHORNE and D.F. STEDMAN	J. Can. Dent. Assoc., July, 1949. 6 pp.

APPENDIX "G"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

PAPERS PUBLISHED

Committee Paper No.	Title and Author	Reference
1	New aspects of periodontal research, by H.K. BOX	Presented at National Convention of Canadian Dentists, Toronto, 29 May, 1946. (App. "C", 5th Mtg. A.C.D.R., 25 Feb., 1947).
2	Concerning the pathogenesis of periodontal disease, by H.K. BOX	1. Periodontology, 19: 11-20. 1948. (App. "G", 7th Mtg. A.C.D.R., 2 Mar., 1948).
3	The effect of various inorganic salts on the solubility of calcium phosphate, tooth enamel and whole teeth in lactic acid, by J.J. RAE and C.T. CLEGG	1. Dent. Res. 27: 52-53. 1948.
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8	The treatment of acute oral Vincent's infection, by W.J. LINGHORNE and D.F. STEDMAN	1. Can. Dent. Assoc., July, 1949. 6 pp.

Committee Paper No.	Title and Author	Reference
9	A comparison of nine local anaesthetics, by H.S. HAMILTON, B.A. WESTFALL and J.K.W. FERGUSON	J. Pharmacol. Expt. Therap. 94: 299-307. 1948.
10	New methods of repairing acrylic dentures without distortion, by D.R. CHRISTIE	J. Can. Dent. Assoc., 15: 582-590, 1949.
11	The corrosion of cobalt chromium alloys in commercial cleaning agents, by D.E.R. COGGLES, O.J. WALKER and H.R. MACLEAN	J. Can. Dent. Assoc. 16: 75-82, 1950.
12	The relation between buffering capacity, viscosity and lacto-bacillus count of saliva, by J.J. RAE and C.T. CLEGG	J. Dent. Res. 28: 589-593. 1949.
13	Mineralization of the growing tooth as shown by Radio-phosphorus autographs (17692), by L.F. BELANGER and C.P. LEBLOND	Proc. Soc. Expt. Biol. Med. 73: 390-391. 1950.
14	Radio-autographic visualization of bone formation in the rat, by C.P. LEBLOND, G.W. WILKINSON, L.F. BELANGER and J. ROBICHON	Am. J. Anat. 86: 2. 1950.
15	Fluoride studies related to the human diet, by MARY P. HAM and M. DOREEN SMITH	Can. J. Research, F, 28: 227-233, 1950.
16	Studies in the regeneration and reattachment of supporting structures of the teeth. I. Soft tissue reattachment, by W.J. LINGHORNE and D.C. O'CONNELL	J. Dent. Res. 29: 419-428. 1950.
17	An examination of the fluoride content of teeth from some Ontario districts, by SHIRLEY R. HARNESS and M. DOREEN SMITH	J. Can. Dent. Assoc., Jan., 1951.

Committee Paper No.	Title and Author	Reference
9	A comparison of nine local anaesthetics, by H.S. HAMILTON, R.A. WESTFALL and A.K.W. FERGUSON	1. Pharmacol. Exptl. Therap. 94: 299-307. 1948.
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17	An examination of the fluoride content of teeth from some Ontario districts, by SHIRLEY R. BARNESS and M. DORSEN SMITH	1. Can. Dent. Assoc., Jan., 1951.

Committee Paper No.	Title and Author	Reference
18	Uptake of radioactive calcium by the dental tissues of the young rat, by A.D'IORIO and J.P. LUSSIER	Rev. Canadienne de Biol. 10: 175-180. 1951.
19	Relining acrylic dentures without distortion, by D.R. CHRISTIE	J. Can. Dent. Assoc., July, 1951.
20	Acrylic Fillings - General comments and some experimental data, by D.R. CHRISTIE	J. Can. Dent. Assoc., 17: 427-435. 1951.
21	Ammonia production in saliva, by R.M. BALLANTYNE, C.T. CLEGG, J.J.RAE, and F.H. LAWFORD	J. of Dent. Research. Vol. 30, No. 3. 1951.

The Secretary of the Committee has a small stock of most of the papers listed and will supply copies to members of the Committee on request.

September, 1951.

Committee Paper No.	Title and Author	Reference
18	Uptake of radioactive calcium by the dental tissues of the young rat, by A.D'IORIO and J.F. LUSSIER	Rev. Canadienne de Biol. 10: 179-180. 1951.
19	Melting acrylic dentures without distortion, by D.R. CHRISTIE	J. Can. Dent. Assoc., July, 1951.
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The Secretary of the Committee has a small stock of most of the papers listed and will supply copies to members of the Committee on request.

September, 1951.

APPENDIX "R"

Suggested Research Projects

By decision of the Committee (Min.4(x), 13th Mtg.) projects suggested as research topics are listed hereunder for consideration by Committee members:

Proposed Subject	Suggested by:	Date
1. Gold inlays over amalgams Little information is available as to whether gold inlays over amalgams cause disintegration of the cement.	Dr. M.H. Garvin	October 1950
2. A study of root canals of teeth This is a very practical research subject as the results would be of value to every practitioner.	Dr. M.H. Garvin	October 1950
3. Social aspects of dental disease	Dr. A.C. Burton	September 1951
4. The effect of light upon foodstuffs in relation to dentistry	Dr. M.H. Garvin	March, 1952

Members of the Associate Committee on Dental Research and its Executive, with their terms of appointment

	Term to Expire
*1. Dr. R. G. Ellis, Chairman, Dean, Faculty of Dentistry, University of Toronto, Toronto, Ont.	31 March, 1954
2. Dr. C. J. Mackenzie, ex officio President, National Research Council, Ottawa, Ont.	---
Representing the Dental Profession	
3. Dr. J. S. Bagnall, Dean, Faculty of Dentistry, Dalhousie University, Halifax, N.S.	31 March, 1952
*4. Dr. E. Charron, Dean of the Faculty of Dental Surgery, University of Montreal, Montreal, Que.	31 March, 1954
5. Dr. M.H. Garvin, Editor-in-Chief, Journal of the Canadian Dental Assoc., 314 Somerset Building, Winnipeg, Man.	31 March, 1953
6. Dr. H.R. MacLean, Lecturer in Operative Dentistry, University of Alberta, Edmonton, Alta.	31 March, 1953
7. Dr. R. H. McDougall, 1505 Hampshire Road, Victoria, B.C.	31 March, 1952
Representing the Royal Canadian Dental Corps	
*8. Brig. E.M. Wansbrough, ex officio Director General of Dental Services, Royal Canadian Dental Corps, Dept. of National Defence, Ottawa, Ont.	---
Representing the Division of Dental Health Department of National Health and Welfare	
9. Dr. H. K. Brown, ex officio, Chief, Dental Health Division, Dept. of National Health and Welfare, Ottawa, Ont.	---

Members of the Associate Committee on Dental Research
and its Executive, with their terms of appointment

		Term to Expire
*1.	Dr. R. G. Gillis, Chairman, Dean, Faculty of Dentistry, University of Toronto, Toronto, Ont.	31 March, 1954
2.	Dr. C. J. Mackenzie, ex officio President, National Research Council, Ottawa, Ont.	---

Representing the Dental Profession

3.	Dr. A. S. Bagnall, Dean, Faculty of Dentistry, Dalhousie University, Halifax, N.S.	31 March, 1952
*4.	Dr. E. Cherrin, Dean of the Faculty of Dental Surgery, University of Montreal, Montreal, Que.	31 March, 1954
5.	Dr. M.E. Garvin, Editor-in-Chief, Journal of the Canadian Dental Assoc., 314 Somerset Building, Winnipeg, Man.	31 March, 1953
6.	Dr. E.R. Jackson, Lecturer in Operative Dentistry, University of Alberta, Edmonton, Alta.	31 March, 1953
7.	Dr. A. N. McDougall, 1505 Hampshire Road, Victoria, B.C.	31 March, 1952

Representing the Royal Canadian Dental Corps

*8.	Brig. E.M. Wansbrough, ex officio Director General of Dental Services, Royal Canadian Dental Corps, Dept. of National Defence, Ottawa, Ont.	---

Representing the Division of Dental Health
Department of National Health and Welfare

9.	Dr. H. K. Brown, ex officio, Chief, Dental Health Division, Dept. of National Health and Welfare, Ottawa, Ont.	---

Representing the Dental Research, Department of Veterans' Affairs

10. Dr. L. A. Kilburn, ex officio Director of Dental Research, Dept. of Veterans' Affairs, Ottawa, Ont.	---

Representing the Basic and Medical Sciences

11. Dr. C. H. Best, Director, Banting and Best Dept. of Medical Research, University of Toronto, Toronto, Ont.	31 March, 1953
12. Dr. A. C. Burton, Head of the Dept. of Biophysics, University of Western Ontario, London, Ont.	31 March, 1954
13. Dr. J.B. Collip, Dean, Faculty of Medicine, University of Western Ontario, London, Ont.	31 March, 1952
14. Dr. A.W. Ham, Professor of Anatomy, Faculty of Medicine, University of Toronto, Toronto, Ont.	31 March, 1952
15. Dr. J.D. Hamilton, Professor of Pathology, University of Toronto, Toronto, Ont.	31 March, 1954
16. Dr. J.B. Macdonald, Assistant Professor of Bacteriology, Faculty of Dentistry, University of Toronto, Toronto, Ont.	31 March, 1954
17. Dr. J.J. Rae, Dept. of Chemistry, University of Toronto, Toronto, Ont.	31 March, 1953
*18. Dr. D.L. Thomson, Chairman of the Dept. of Biochemistry, McGill University, Montreal, Que.	31 March, 1953
*19. Mr. S. J. Cook, Secretary Public Relations Branch, National Research Council, Ottawa, Ont.	---

* Members of the Executive.

Representing the Dental Research,
Department of External Affairs.

10. Dr. L. A. Tilburn, ex officio
Director of Dental Research,
Dept. of External Affairs,
Ottawa, Ont.

Representing the Basic and Medical Sciences

11. Dr. O. M. Post,
Director, Banting and Best Dept. of
Medical Research,
University of Toronto,
Toronto, Ont.

31 March, 1953

12. Dr. A. C. Burton,
Dean of the Dept. of Biophysics,
University of Western Ontario,
London, Ont.

31 March, 1953

13. Dr. J.C. Calitz,
Dean, Faculty of Medicine,
University of Western Ontario,
London, Ont.

31 March, 1952

14. Dr. A.W. Hoo,
Professor of Anatomy,
Faculty of Medicine,
University of Toronto,
Toronto, Ont.

31 March, 1952

15. Dr. J.E. Hamilton,
Professor of Pathology,
University of Toronto,
Toronto, Ont.

31 March, 1954

16. Dr. J.D. Macdonald,
Assistant Professor of Bacteriology,
Faculty of Dentistry,
University of Toronto,
Toronto, Ont.

31 March, 1954

17. Dr. W.O. Mac,
Dept. of Chemistry,
University of Toronto,
Toronto, Ont.

31 March, 1953

*18. Dr. D.L. Thomson,
Chairman of the Dept. of Biochemistry,
McGill University,
Montreal, Que.

31 March, 1953

*19. Mr. S. L. Cook, Secretary
Public Relations Branch,
National Research Council,
Ottawa, Ont.

* Members of the Executive.

INITIAL DISTRIBUTION

(i) To members of the

Associate Committee on Dental Research

1. Dr. R.G. Ellis, Chairman
2. Dr. C.H. Best
3. Dr. J.S. Bagnall
4. Dr. H.K. Brown
5. Dr. A.C. Burton
6. Dr. E. Charron
7. Dr. J.B. Collip
8. Dr. M.H. Garvin
9. Dr. A.W. Ham
10. Dr. J.D. Hamilton
11. Dr. L.A. Kilburn
12. Dr. J.B. Macdonald
13. Dr. C.J. Mackenzie
14. Dr. H.R. MacLean
15. Dr. R.H. McDougall
16. Dr. J.J. Rae
17. Dr. D.L. Thomson
18. Brig. E.M. Wansbrough
19. Mr. S.J. Cook, Secretary

(ii) To other persons:-

20.-21. Deans of Dental Schools (who are not members of the Committee)

20. Alberta - Dr. W.S. Hamilton

21. McGill - Dr. D. Prescott Mowry

22. Master Copy (General Secretary)

23. Board Room Copy

24. Library

25-30 Reserve.

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COPY NO. 1

NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS

OF THE

SIXTEENTH MEETING

OF THE

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

OTTAWA

29 SEPTEMBER 1952

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Appendix "C" Oral pathology and bacteriology,Fellowship grant, (Dr. Gerald Shklar)

Appendix "D" The Effect of the endocrines on the teeth and jaws, DR 38

Appendix "E" Bacteriology of periodontal disease, DR 35

Appendix "F" Chemical mechanisms in dental caries, DR 1

Appendix "G" Demineralization of hard tissues as bone and teeth by organic chelating agents, DR 36

Appendix "H" Studies in the chemistry of the saliva, DR 42

Appendix "I" Further studies in the electromyography of the head, face and neck, DR 37

Appendix "J" A study of the normal and abnormal physiologic forces of the oral cavity, DR 26

Appendix "K" Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radio-active elements, DR 23

Appendix "L" Sectioning of teeth and surrounding structures and the effects of potassium deficiency and of pyridoxine deficiency on the teeth and surrounding structures of dogs, DR 39

Appendix "M" Development of a method for determining fluorine by means of a photoelectric colorimeter, DR 28

Appendix "N" A study of migration of tooth germs before and during mixed dentition, Fellowship grant, (Dr. Willa Liu Chou)

Appendix "O" A comparison of the patterns of clinical eruption of the deciduous teeth in man with growth in width of the dental arches by Margaret E. Hatton (Summary and conclusion, from a thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy at the University of Toronto, 1952)

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Initial Distribution.

NATIONAL RESEARCH COUNCIL

Proceedings

of the

Sixteenth Meeting

of the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in Council Chamber, National Research Building
9:30 a.m., Monday, 29 September, 1952.

---

1. Attendance

Dr. R. G. Ellis, Chairman
Dr. E.W.R. Steacie, President NRC
Dr. J. S. Bagnall
Dr. H. K. Brown
Dr. A. C. Burton
Dr. E. Charron
Dr. M. H. Garvin
Dr. J. D. Hamilton
Dr. L. A. Kilburn
Dr. J. B. Macdonald
Dr. R. A. McDougall
Dr. E. Pagé
Dr. J. J. Rae
Dr. D. L. Thomson
Brig. E. M. Wansbrough

Mr. S. J. Cook, Secretary

By Invitation

Dr. L. A. Gill, representing Dr. A. G. Racey
Mr. R. C. Greulich, representing Dr. C. P. Leblond
Dr. R. E. Moyers
Mr. B. P. Scull, Information Services

Apologies for their absence were received from - Dr. C.H.Best and Dr. H. R. MacLean.

2. Chairman's Remarks

THE CHAIRMAN thanked the Committee members for the telegram they had so kindly sent to him during the March meeting which he had unfortunately been unable to attend because of illness. He also expressed his appreciation to Dr. D.L. Thomson who had so ably presided on that occasion. The Chairman welcomed President E.W.R. Steacie who was attending a meeting of this Committee for the first

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time and said that he hoped the President would find it possible to follow the work of the Committee closely. He mentioned that Committee members had received much helpful advice from Dr. C. J. Mackenzie during his term of office. He hoped that the Committee by its work would continue to merit the interest and guidance of the President.

He also welcomed the newly appointed member Dr. E. Pagé and the reappointed members - Dr. J. S. Bagnall, Dr. A. W. Ham and Dr. R. H. McDougall.

Invitations had been sent to certain grantees and to the fellowship holders to be present at this meeting and to report on their work. Dr. Racey had been unable to come but had asked Dr. L.A. Gill to act on his behalf. Dr. Moyers was present and Dr. Leblond was represented by Mr. R.C. Greulich. Neither of the two fellowship holders, Dr. Shklar or Dr. Willa Liu Chou had been able to come to this meeting although both had been invited.

3. Minutes of the 15th Meeting

It was MOVED by Dr. Charron and SECONDED by Dr. Bagnall,

That the Minutes of the 15th Meeting, having been circulated, be taken as read and approved. CARRIED.

4. Business Arising Out of the Minutes

(i) Dr. Bagnall's Bibliography (Min. 6(iii) 15th Mtg.)

THE SECRETARY reported that of the 320 volumes purchased, 220 had now been distributed including 37 complimentary copies and 183 sold as at September 1952. Books had been sent to fourteen different countries including the United States where 132 copies had been sold and 9 complimentary copies distributed to 25 different States. There were still 100 copies on hand.

THE CHAIRMAN said that the American College of Dentists had included, in their Journal, a very complimentary review of Dr. Bagnall's book noting that "It is a job well done".

A further mark of appreciation for this work from the same organization was contained in a letter to the Secretary advising him that it is the custom of the American College of Dentists to place a memorial book in the dental library of each deceased member's Alma Mater and that Dr. Bagnall's book had been selected as their choice for this purpose in 1952. Ten copies had subsequently been purchased by the College.

THE CHAIRMAN said that the action taken by the American College of Dentists was a high tribute to the author, and that it was gratifying to the members of the Committee to see Dr. Bagnall's work honoured in this way.

time and said that he hoped the President would find it possible to follow the work of the Committee closely. He mentioned that Committee members had received much helpful advice from Dr. C. J. Mackenzie during his term of office. He hoped that the Committee by its work would continue to merit the interest and guidance of the President.

He also welcomed the newly appointed member Dr. E. Page and the reappointed members - Dr. J. S. Bagnall, Dr. A. W. Ham and Dr. R. H. McDougall.

Invitations had been sent to certain grantees and to the fellowship holders to be present at this meeting and to report on their work. Dr. Racey had been unable to come but had asked Dr. L.A. Gill to act on his behalf. Dr. Moyers was present and Dr. Lebland was represented by Mr. R.C. Greulich. Neither of the two fellowship holders, Dr. Shklar or Dr. Willa Liu Chou had been able to come to this meeting although both had been invited.

3. Minutes of the 15th Meeting

It was MOVED by Dr. Charron and SECONDED by Dr. Bagnall,

That the Minutes of the 15th Meeting, having been circulated, be taken as read and approved. CARRIED.

4. Business Arising Out of the Minutes

(1) Dr. Bagnall's Bibliography (Min. 6(111) 15th Mtg.)

THE SECRETARY reported that of the 320 volumes purchased, 220 had now been distributed including 37 complimentary copies and 183 sold as at September 1952. Books had been sent to fourteen different countries including the United States where 132 copies had been sold and 9 complimentary copies distributed to 25 different States. There were still 100 copies on hand.

THE CHAIRMAN said that the American College of Dentists had included, in their Journal, a very complimentary review of Dr. Bagnall's book noting that "It is a job well done".

A further mark of appreciation for this work from the same organization was contained in a letter to the Secretary advising him that it is the custom of the American College of Dentists to place a memorial book in the dental library of each deceased member's Alma Mater and that Dr. Bagnall's book had been selected as their choice for this purpose in 1952. Ten copies had subsequently been purchased by the College.

THE CHAIRMAN said that the action taken by the American College of Dentists was a high tribute to the author, and that it was gratifying to the members of the Committee to see Dr. Bagnall's work honoured in this way.

(ii) Honour to Dr. Moyers (Min. 19, 15th Mtg.)

THE CHAIRMAN said that in the proceedings of the 15th Meeting the Secretary had included a note regarding an honour received by Dr. Moyers and he thought this note might also be included in the current proceedings together with an expression of congratulations from the Committee to Dr. Moyers in this connection. The note read as follows:

> "On March 5, 1952, a news item reported that Dr. Moyers had been presented with the Golden Cross of Phoenix 'for services rendered to Greece during the enemy occupation and after'. He parachuted into the mountains of the province of Evrytania, Greece, during the German occupation of World War II. He acted as liaison between the Resistance Movement in Greece and the Near East Allied Command. While there, he established the first Health Unit which the Resistance Movement lacked entirely."
>
> The Committee received this news with enthusiasm.

(iii) Membership of Committee (Min. 30, 15th Mtg.)

THE CHAIRMAN reported officially that three members of the Committee, Drs. Bagnall, Ham and McDougall, had been reappointed as members of the Associate Committee on Dental Research for a further term of three years, to 31 March 1955 and that Dr. E. Pagé, Faculty of Medicine, Laval University, had also been appointed for a first term of three years to the same date.

(iv) Thesis by Dr. Margaret E. Hatton (Min. 6, 15th Mtg.)

THE SECRETARY laid on the table for inspection a copy of a thesis submitted by Margaret Elizabeth Hatton in conformity with the requirements for the degree of Doctor of Philosophy at the University of Toronto, 1952. The subject of the thesis was "A Comparison of the Patterns of Clinical Eruption of the Deciduous Teeth in Man with Growth in Width of the Dental Arches".

> On a motion by Dr. J. J. Rae, SECONDED by Dr. Thomson,
>
> It was AGREED that the summary and conclusion in the thesis should be included in the Committee's proceedings. (See Appendix "O")

Consideration of Reports from Grantees

5. DR 1 - Dr. J. J. Rae

"Chemical mechanisms in dental caries"

DR. RAE presented a summary and interim report No. 14 on his project, both of which appear in APPENDIX "F"

(14) Honour to Dr. Moyers (Min. 19, 15th Mtg.)

THE CHAIRMAN said that in the proceedings of the 15th Meeting the Secretary had included a note regarding an honour received by Dr. Moyers and he thought this note might also be included in the current proceedings together with an expression of congratulations from the Committee to Dr. Moyers in this connection. The note read as follows:

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The Committee received this news with enthusiasm.

(14) Membership of Committee (Min. 20, 15th Mtg.)

THE CHAIRMAN reported officially that three members of the Committee, Drs. Bagnall, Ham and McDougall, had been reappointed as members of the Associate Committee on Dental Research for a further term of three years, to 31 March 1955 and that Dr. E. Page, Faculty of Medicine, Laval University, had also been appointed for a first term of three years to the same date.

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THE SECRETARY laid on the table for inspection a copy of a thesis submitted by Margaret Elizabeth Hatton in conformity with the requirements for the degree of Doctor of Philosophy at the University of Toronto, 1952. The subject of the thesis was "A Comparison of the Patterns of Clinical Eruption of the Deciduous Teeth in Man with Growth in Width of the Dental Arches".

On a motion by Dr. J. J. Rae, SECONDED by Dr. Thomson,

It was AGREED that the summary and conclusion in the thesis should be included in the Committee's proceedings. (See Appendix "O")

Consideration of Reports from Grantees

5. DR I - Dr. J. J. Rae

"Chemical mechanisms in dental caries"

DR. RAE presented a summary and Interim report No. 14 on his project, both of which appear in APPENDIX "P"

He drew attention to a slight error in the typing of his report. In the last line of para. 3 (2) on page F-2, the figure should read "4.7 gamma of solids per ml." instead of "4.78 of solids per ml.". He said that the error probably arose from the way in which he made the Greek letter so that it looked like 8.

THE SECRETARY said that this correction would be made in the proceedings.

THE CHAIRMAN observed that at least five papers were listed in the Committee proceedings as having arisen out of this project which he thought was a very satisfactory record.

6. DR 13 - Dr. M. Doreen Smith

"Fluoride content of enamel and dentin of teeth from Toronto children studied in relation to pablum history of the children's diets"

THE CHAIRMAN said that Dr. Smith had been invited to attend this meeting but that as she had spent the last few months in Africa and did not get back to the University until 18 September, she had been unable to complete a report. She had asked leave to postpone her presentation until the Spring meeting which he thought was a wise decision. Dr. Smith had sent the following note:-

"....

Two very competent graduate students undertook the collection and analysis of teeth during the summer months. They had completed the separation and analysis of over 50 teeth, but although one of the workers, Mr. Panalaks, carried on considerable correspondence with Ottawa and Sarnia to get teeth, the samples from Sarnia have not been forthcoming and are needed to complete the report. Dr. Storey of Sarnia was good enough to call on us the other day and tell us of the effort made by himself and his son to get these teeth; he suggests that an official letter from Dr. H. K. Brown might bring results. Dr. Storey will provide us with the names of the dentists and we shall try this as a last resort.

.....

" Miss M.P. Ham and I are also working on a paper for publication on her study on the "Absorption of Fluorine by Humans", which I think should be of interest to the Committee. This will also be submitted on completion.

" I am sorry not to have my report ready and am afraid I neglected to foresee this situation when I planned my leave of absence last spring. I wish to thank the Committee for the invitation to attend the meeting and regret that I cannot accept."

7. DR 22 - Dr. C. H. Best

"Part 1 - An investigation of the mechanism of repair of the supporting structures of the teeth;
Part 2 - Fusospirochetal infection and pre-existing inflammation"

THE CHAIRMAN said that Dr. Best was at present in Australia and that a report on his project had been prepared by Dr. W.J. Linghorne. This report was in the mail but unfortunately had not yet been received.

(Secretary's note)- The summary and report on this project were received the day after the meeting and have been included in these proceedings as APPENDIX "P"

8. DR 23 - Dr. C. P. Leblond

"Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radioactive elements"

In the absence of Dr. Leblond the report on this project was presented by Mr. R. C. Greulich.

MR. GREULICH presented the following reports: (i) Summary report on the project by Dr. C.P. Leblond; (ii) Entry of radio-carbon into the teeth by R. C. Greulich; (iii) Entry of radio-carbon into the organic matrix of growing teeth by R. C. Greulich; (iv) Development of rat teeth studied with calcium 45 by Y. Kumamoto; (v) Sectioning of adult teeth by Mr. M. Weiss. All of foregoing reports appear in APPENDIX "K"

DR. BURTON pointed out that Dr. Bélanger at the University of Ottawa is working in a similar field and he thought it would be important for the Committee to keep in touch with all Canadian workers interested in this subject.

THE CHAIRMAN added that there are now three workers, namely, Dr.Leblond, Dr. Lussier, and Dr. Bélanger who are all engaged in similar projects.

DR. BURTON suggested that Dr. Bélanger might be encouraged to seek the assistance of the Committee in his work.

9. DR 26 - Dr. R. E. Moyers (project completed)

"A study of the normal and abnormal physiologic forces of the oral cavity"

DR. MOYERS stated that although this project had now been completed, some additional work had recently been done under his direction using the apparatus which had been provided under the grant. He presented a further summary which appears in APPENDIX "J"

THE CHAIRMAN observed that it was gratifying to know that equipment purchased for a specific purpose was being found useful in further work.

10. DR 28 - Dr. O. J. Walker

"Development of a method for determining fluorine by means of a photo-electric colorimeter"

THE CHAIRMAN said that Dr. Best was at present in Australia and that a report on his project had been prepared by Dr. W.J. Linghorne. This report was in the mail but unfortunately had not yet been received.

(Secretary's note):- The summary and report on this project were received the day after the meeting and have been included in these proceedings as APPENDIX "p"

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"A study of the normal and abnormal physiologic forces of the oral cavity"

DR. MOYERS stated that although this project had now been completed, some additional work had recently been done under his direction using the apparatus which had been provided under the grant. He presented a further summary which appears in APPENDIX "l"

THE CHAIRMAN observed that it was gratifying to know that equipment purchased for a specific purpose was being found useful in further work.

10. DR 28 - Dr. O. J. Walker

"Development of a method for determining fluorine by means of a photo-electric colorimeter"

THE CHAIRMAN presented the summary and report submitted by Dr.Walker which appear in full in APPENDIX "M"

11. DR 35 - Dr. J.B. Macdonald

"Bacteriology of periodontal disease. Experimental fusospirochetal infection with mixtures of pure cultures"

The summary and report on this project, presented by Dr. J.B. Macdonald appear in APPENDIX "E"

THE CHAIRMAN said that a letter had been received from Dr. Macdonald requesting the support of the Committee in the publication of a monograph entitled "The motile non-sporulating anaerobic rods of the oral cavity" in which the material presented in the report received at this meeting is to be published. He suggested that discussion of this request be deferred until other matters of a financial nature were being considered. (See Min. 23)

12. DR 36 - Dr. G. Nikiforuk

"Demineralization of hard tissues as bone and teeth by organic chelating agents"

At the request of the Chairman this report was presented by Dr. Macdonald. The summary and report appear in APPENDIX "G"

It was suggested that Dr. Nikiforuk or Dr. Hunter be invited to attend the next meeting to present the results of their work in person.

DR. MACDONALD said that the first report of Dr. Nikiforuk's work on this subject appeared in SCIENCE about two years ago. Since then at least one other report has been published. The present report presents quantitative data whereas the earlier publications merely indicated that it was possible to effect demineralization by the process described.

The following suggestions regarding possible desirable changes in the text of the report were made:

(i) Page 3 - line 2. After "figure 1" add "which is taken from Technical Bulletin No. 2, Bersworth Chemical Company"

(ii) Page 7, para. 1, line 6 - Dr. Thomson suggested that the sentence be deleted which reads: "This interesting fact is explained by the observation that solubility product constant of bone decreases as the pH increases". AGREED.

(iii) Page 3, para. 3, line 3 - Dr. Rae said that he would prefer to see the term "hydrogen ion" used instead of the word "protons"

(iv) Fig. 4 - Dr. Pagé pointed out that it was rather unusual to put "time" on the vertical scale of a chart.

DR. HAMILTON inquired whether Dr. Nikiforuk would be willing to undertake a much broader project. He thought there was a great field of study available in examining all methods that are being suggested for demineralization of bone specimens.

13. DR 37 - Dr. R. E. Moyers

"Further studies in the electromyography of the head, face and neck"

DR. MOYERS presented a report on this project of which a summary appears in APPENDIX "I"

DR. BURTON observed that he has a student working on the relation between load and electrical activity in the muscular operation of the forearm. He thought this problem was somewhat related to the one reviewed by Dr. Moyers.

14. DR 38 - Dr. K.J. Paynter

"The effect of the endocrines on the teeth and jaws:
(I) The effect of thiouracil on the development of molar teeth of rats,
(II)The effect of thiouracil on the bone of the jaws of rats"

The summary and report on this project, presented by Dr. Macdonald, appear in APPENDIX "D"

DR. MACDONALD pointed out that this report emphasizes the importance of the time factors i.e. that all processes were slowed down at proportional rates.

DR. BURTON suggested that radioactive iodine might be used to depress thyroid.

THE CHAIRMAN recalled that the use of thiouracil was adopted on the basis of work done at Columbia University.

15. DR 39 - Dr. A.G. Racey

"Sectioning of teeth and surrounding structures and the effects of potassium deficiency and of pyridoxine deficiency on the teeth and surrounding structures of dogs"

DR. L.A. GILL, representing Dr. Racey, presented the summary of work under this project which appears in APPENDIX "L"

16. DR 42 - Dr. G. Nikiforuk

"Studies in the chemistry of the saliva. II. The reducing property of saliva towards some oxidation-reduction indicators"

THE SECRETARY reported that the subtitle of this project had been changed at the request of Dr. Nikiforuk from "II. The reduction of methylene blue by saliva" to the more general subject "II. The reducing property of saliva towards some oxidation-reduction indicators".

DR. MACDONALD said that Dr. Nikiforuk had followed up suggestions made to him at the last meeting of the Committee but that he would ask the indulgence of the Committee for longer time before he defines the objectives of his present work. By next March he should be able to comment satisfactorily on this subject.

The summary of the work appears in APPENDIX "H"

DR. THOMSON thought that a method used for determining metabolic processes of total bacterial populations might arise from this project.

DR. RAE inquired whether any attempt had been made to withdraw saliva directly from the gland.

DR. MACDONALD said that Dr. Nikiforuk had tried on several occasions to do this but had found it very difficult to achieve.

DR. RAE suggested that in item 3 of the summary on page 1, it would be helpful if the rate and duration of centrifuging were shown.

17. DR 43 - Drs. S.G. Davis and H.R. MacLean

"Electro potentials between dental alloys"

THE CHAIRMAN read the brief summary of this project which appears in APPENDIX "B"

18. Appreciation of Reports

DR. THOMSON said that the reports presented at this meeting of work done by grantees set a very high standard of quality.

THE CHAIRMAN remarked that at luncheon there had been some discussion as to the utility of mid-year meetings for the reading of reports as often work is not sufficiently advanced to permit the presentation of a comprehensive statement. He agreed with Dr. Thomson's appraisal of the reports presented at this meeting but suggested that the general question as to whether two meetings a year should be held, might be reviewed at the next meeting of the Committee.

19. List of Committee Projects

THE CHAIRMAN suggested and it was AGREED, that a complete list of projects sponsored by the Committee should be included as an Appendix to the proceedings of each meeting for convenience of reference. It was agreed that the listing should show the project number, name of the grantee, the department and institution at which he works, and the title of his project. It should also be indicated whether the project has been completed or is in progress.

20. Fellowships (Min. 24, 15th Mtg.)

(i) Dr. Gerald Shklar

THE CHAIRMAN reported that Dr. Shklar had completed his fellowship under the Dental Committee at Tufts College Dental School, Boston, Mass., working with Professor Irving Glickman of the Department of Oral Pathology and Periodontology. Dr. Shklar had been invited to attend this meeting and had planned to do so but unforeseen circumstances had intervened.

DR. SHKLAR had submitted a summary and a report which appear in APPENDIX "C" He had also forwarded, for the information of the Committee, a copy of his thesis entitled "The periodontium and other oral tissues in the alarm reaction" which he had submitted in partial fulfilment of the requirements for the degree Master of Science at Tufts May 1952. Dr. Shklar had requested that his thesis be returned to him but as it seemed desirable to have a copy in the Dental Committee records, the Secretary had undertaken to have copies made for this purpose.

THE CHAIRMAN said that a letter had been received from Dr. Irving Glickman and as this letter was of interest to members of the Committee it is here included:-

Letter dated 28 August 1952 from Dr. Irving Glickman,
Professor of Oral Pathology and Periodontology,
Tufts College, Dental School,
136 Harrison Avenue,
Boston, 11, Mass., U.S.A.

Re: Dr. Gerald Shklar

" I am happy to be able to give you a favourable report regarding the activities of Dr. Gerald Shklar during the past year when he was a recipient of your fellowship. Dr. Shklar is a courteous, industrious young man with a natural interest in dental science plus the zest to pursue that interest. He has successfully completed the requirements for the Mater of Science Degree which was awarded him by Tufts College in June 1952. His research as part of the requirements for the Master of Science Degree was thoroughly conducted. I suggested that he submit a copy of his thesis, The Periodontium and Other Oral Tissues in the 'Alarm Reaction' to you.

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" In addition to devoting himself to this major project, Dr. Shklar has been interested in research dealing with the bacteriology of gingival disease and also the effect of adrenalectomy on the periodontal tissues of the albino rat.

" He has also been engaged in clinical activities which include the management of periodontal disease as well as participation in a clinic devoted to oral medicine. I invited Dr. Shklar to remain here at Tufts as an instructor in oral pathology and periodontology for the current academic year which he has accepted.

" During this year, I hope to be able to succeed in tempering his enthusiasm with a somewhat keener analytical sense. In his anxiety to create a good impression by his accomplishments, he sometimes tends to be less critical than he might be. This is quite understandable at this stage in his development."

DR. BURTON commented that the first report by Dr. Shklar was an excellent example of concise, clear, scientific writing.

DR. THOMSON observed that since Dr. Ham is working in the same field he should be advised of Dr. Shklar's work.

DR. MACDONALD said that in regard to the second study made by Dr.G. Shklar he was surprised to see that hypersensitivity studies were being done on disease-free guinea pigs. He thought that human subjects should have been used and it was not surprising that Dr.Shklar got only negative results from the use of animals which are not subject to gingival disease.

(ii) Dr. Willa Liu Chou

"A study of migration of tooth germs before and during mixed dentition"

THE CHAIRMAN presented a summary of the work which had been done by Dr. Willa Liu Chou under her fellowship grant. This report appears in APPENDIX "N"

THE CHAIRMAN asked Dr. Moyers if he would care to comment on this study.

DR. MOYERS explained that the work under the fellowship had been impeded because of Dr. Liu's failure to obtain a United States' visa which would permit her to go to Cleveland and Iowa City to make tracings of records that are available in these two centres and which she had intended to use in the furtherance of her investigation under the fellowship award granted to her by the Committee. He explained that she had come out to Canada from Hong Kong and that despite the fact that he had made arrangements for her to live in both Cleveland and Iowa City with friends of his, the immigration authorities in the United States had prevented her from entering that country. He said that Dr. Liu is very capable, highly trained and has worked out the necessary techniques but the material she requires for study is only available in the United States and it cannot be borrowed. Similar growth studies have been

started at Burlington, Ontario, but it will be more than ten years before any usable data become available. He felt that Dr. Liu was extremely conscientious and said that she even hesitated to accept funds from the Committee for this work so long as she was unable to obtain a visa to enter the United States.

DR. BURTON asked whether it would not be possible to employ someone to make the drawings and measurements that Dr. Liu wants and send this person to the United States for that purpose.

DR. MOYERS said that he thought it would be quite possible to find and train a student worker who could do such a job and bring back the material wanted by Dr. Liu.

After some further discussion, it was MOVED by Dr. Burton and SECONDED by Dr. Rae,

(i) That in order to obtain from United States sources the tracings and measurements required by Dr. Willa Liu Chou to complete the study she has in progress under a fellowship award from the Associate Committee on Dental Research, Dr. Moyers be asked to search for a person of suitable qualifications who could be trained by Dr. Liu and sent to Cleveland and Iowa City to secure the information needed in this study;

AND

(ii) That the Executive be authorized to provide the necessary funds for this purpose from the Committee appropriation, and to make all other arrangements that they may consider essential for the completion of this project. CARRIED.

21. Finances

(i) Financial standing of the Committee, September, 1952

THE CHAIRMAN asked for a statement of Dental Committee's finances as of September 1952 and the Secretary supplied the following information:-

Dental Committee Finances, Sept.1952

1. Total Grant, 1952-53			$40,700.00
2. Carried over from 1951-52 for purchase of goods ordered but not delivered on 1 April, 1952			349.77
3. Grants awarded 1952-53			21,390.00
4. Total available to grantees			21,739.77
5. Less funds in the possession of grantees, 1 April, 1952			2,597.14
6.		Net	19,142.63
7. Advanced to grantees to September 1952			7,850.33
8. Balance payable to grantees			11,292.30
9. Other expenditures and commitments:			
(Travel	$236.01		
Contingencies	14.72)	250.73	
Balance due on Dr. Liu's Fellowship award		220.00	
		470.73	470.73
10. Unexpended balance available for disposition by the Committee			
(1+2-5) - (6+9) = 10			18,839.27

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10. Unexpended balance available for disposition by the Committee (1+2-5) - (6+9) = 10		18,839.27

(ii) Estimates, 1953-54

THE SECRETARY said, that, pending confirmation by the Associate Committee on Dental Research, he had submitted tentative estimates to cover the Committee's requirements in 1953-54 as follows:-

Associate Committee on Dental Research

Grants in aid of research	$32,500
Fellowships	5,000
Committee Travel	2,500
Printing of Proceedings, etc.	200
Contingencies	500
	$40,700

THE CHAIRMAN pointed out that estimates for Committees have to be submitted each year in September and that the Secretary had no choice but to propose the same amounts as had been provided in the previous year. He thought the Committee should give some consideration to its requirements and authorize in advance a statement which the Secretary could use in the autumn when he was asked for the estimates. He suggested and it was AGREED that the estimates for 1954-55 should be put down as an item on the Agenda for the Spring meeting 1953.

The estimates as prepared and submitted by the Secretary for 1953-54 were APPROVED.

22. Applications for grants, 1952-53 (Min. 31, 15th Mtg.)

A few new applications for grants for the current year were received and considered and action was taken on them as follows:

DR 44	Dr. Arthur Harry Craven, Lecturer in Orthodontics, McGill University Dental School, Montreal, Que.	The growth in width of the face and head of the Macaque monkey as revealed by vital staining.	$600

This application was submitted by Dr. D. L. Thomson. He said that the purpose of this investigation was to obtain further information on the growth sites contributing to increase in width of the facial and cranio-facial structures. An article by Moore in the American Journal of Orthodontics had already dealt with the technique and work in one plane. The candidate wishes to work in another plane using the same technique. He proposed to use two monkeys but was asking that provision be made for the purchase of four monkeys as a protective measure.

DR. MOYERS said that the work previously done by Moore was very good and he thought the proposed project was excellent.

(11) Estimates, 1953-54

THE SECRETARY said, that, pending confirmation by the Associate Committee on Dental Research, he had submitted tentative estimates to cover the Committee's requirements in 1953-54 as follows:-

Associate Committee on Dental Research

Grants in aid of research	$32,500
Fellowships	5,000
Committee Travel	2,300
Printing of Proceedings, etc.	200
Contingencies	500
	$40,500

THE CHAIRMAN pointed out that estimates for Committees have to be submitted each year in September and that the Secretary had no choice but to propose the same amounts as had been provided in the previous year. He thought the Committee should give some consideration to its requirements and authorize in advance a statement which the Secretary could use in the autumn when he was asked for the estimates. He suggested and it was AGREED that the estimates for 1954-55 should be put down as an item on the Agenda for the Spring meeting 1953.

The estimates as prepared and submitted by the Secretary for 1953-54 were APPROVED.

22. Applications for grants, 1952-53 (Min. 31, 15th Mtg.)

A few new applications for grants for the current year were received and considered and action was taken on them as follows:

DR 44	Dr. Arthur Harry Craven, Lecturer in Orthodontics, McGill University Dental School, Montreal, Que.	The growth in width of the face and head of the Macaque monkey as revealed by vital staining. $600

This application was submitted by Dr. D. I. Thomson. He said that the purpose of this investigation was to obtain further information on the growth sites contributing to increase in width of the facial and cranio-facial structures. An article by Moore in the American Journal of Orthodontics had already dealt with the technique and work in one plane. The candidate wishes to work in another plane using the same technique. He proposed to use two monkeys but was asking that provision be made for the purchase of four monkeys as a protective measure.

DR. MOYERS said that the work previously done by Moore was very good and he thought the proposed project was excellent.

DR. THOMSON thought this project could be completed during the present year and that the candidate might then be disposed to file a further application with the Committee for work next year.

It was MOVED by Dr. Thomson and SECONDED by Dr.Hamilton,

That a grant under DR 44 be made to Dr. Craven in the amount of $600. APPROVED.

DR 45 Dr. R. E. Moyers, Prof. and Head, Dept.of Orthodontics, Faculty of Dentistry, University of Toronto, Toronto, Ont. — Study of the incidence of malocclusion — $1700

The amount requested under this grant was $2100 including $1900 to cover the salary of a research assistant for six months.

DR. MOYERS said that The National Health Grant is supporting a long-term study on the interception of malocclusion, at Burlington, Ontario, within the framework of the Halton County Health Unit. In the present project it was proposed to study with great care the original survey records using the technique developed by Dr. William Elsasser so that a base level for the greater study might be established. Dr. Margaret Hatton will be available to work on this project and her experience and previous research make her uniquely suited for this task.

Some discussion ensued as to whether malocclusion is a dental research problem or a clinical public health study, and it was agreed that the proposed investigation is not fundamental research of a kind that the Committee has been supporting. Nevertheless, it was thought that the subject was important and that it might be useful to support it initially in the hope that the Public Health Services might take it up and carry it forward at a later date.

It was AGREED however, that the provision for the salary of a research assistant should not exceed a rate of $3,000 per year which is the amount offered to a single person under the Committee's fellowship plan.

It was MOVED by Dr. Macdonald and SECONDED by Dr. Garvin

That the application from Dr. R.E. Moyers under DR 45 be amended by reducing the provision for salaries from $1900 to $1500 making the total amount of the grant $1700, and

That it be suggested to the grantee that he make an effort to arrange for the provision of further support for this investigation after the end of the present fiscal year through the funds provided under the Public Health Grant. CARRIED.
(DR. J. J. RAE dissenting)

DR 46 Dr. R. E. Moyers, Prof. and Head, Dept. of Orthodontics, Faculty of Dentistry, University of Toronto, Toronto, Ont. — An analysis of treated orthodontic cases

There was some discussion regarding the items for "shelving and boxes and other supplies" mentioned in the application, it being thought that it might properly be expected that these items should be provided by the University.

It was MOVED by Dr. Macdonald and SECONDED by Dr. McDougall,

That the application by Dr. R. E. Moyers be approved insofar as the salary provision for the technician is concerned, namely $520 but that no provision be made under the grant for the purchase of supplies and equipment for which $864 had been asked. TOTAL APPROVED: $520

DR 47 Dr. H. A. Hunter, Associate Prof. of Dentistry, University of Toronto, Toronto, Ont. — The pathological characteristic of experimental fuso-spirochetal infections. $1200

DR. MACDONALD explained that the proposed project provides for the integration of new results on results already known and is supplementary to a project previously held by Dr. Hunter. He has been on a teaching program recently which left him little time for research but now he finds that he can take up research work again.

It was MOVED by Dr. Thomson and SECONDED by Brig. E.M. Wansbrough,

That the application of Dr. H.A. Hunter for a grant under DR 47 be APPROVED in the amount of $1200 CARRIED.

23. Publication of a Monograph (see Min. 10)

THE CHAIRMAN said a letter had been received from Dr. J.B.Macdonald requesting that the Associate Committee on Dental Research give consideration to publishing results of certain studies which he had made under a National Research Council grant. Title of the proposed monograph is "The motile non-sporulating anaerobic rods of the oral cavity". The manuscript consists of 120 pages of double-spaced typing and includes tables and ten plates. Excerpts from this manuscript have been presented to the Committee in the semi-annual report shown in Appendix "E".

DR. MACDONALD had suggested that it would be desirable to have all the results of his investigation assembled in one place rather than in a series of papers since only one subject is involved.

There was considerable discussion.

DR. RAE thought it might be published in a scientific journal concerned with microbiology.

DR. THOMSON suggested that Bacteriological Reviews might be willing to publish the article in a series of sections.

BRIG. WANSBROUGH concurred in the view that publication of this manuscript should be offered to journals in which it was likely that the text would be read.

DR. THOMSON added that the proposed method of issue offered very poor facilities for the distribution of the text. He suggested that Philosophical Transactions of the Royal Society of London might provide a suitable outlet. He was sure that Dr. Macdonald would do well to explore the field of publications to which libraries generally subscribe.

It was MOVED by Dr. Hamilton and SECONDED by Dr. Thomson,

That the Associate Committee on Dental Research, having carefully reviewed the application of Dr. J.B. Macdonald, for a grant-in-aid of the publication of the monograph entitled "The motile non-sporulating anaerobic rods of the oral cavity", suggests that the applicant first explore the possibility of having his manuscript published in some scientific journal that has a wide distribution in scientific libraries rather than to publish it as a booklet that might have a very restricted distribution, and

That, subsequently, if Dr. Macdonald finds that publication is only possible if a subvention of some kind is made, the Committee would then entertain an application for such assistance, since it is believed that a useful purpose would be served in assembling this valuable material in one place for convenient reference. CARRIED.

24. Suggested Research Projects (Min.33,15th Mtg.)

DR. GARVIN inquired whether deans of dental schools were informed of the list of suggested research projects now being included as a standing appendix in the proceedings of the Committee.

THE CHAIRMAN said that copies of the proceedings are sent to the deans of all Canadian dental schools but that he thought it would be useful if the Secretary were to send a note to each of them

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That, subsequently, if Dr. Macdonald finds that publication is only possible if a subvention of some kind is made, the Committee would then entertain an application for such assistance, since it is believed that a useful purpose would be served in assembling this valuable material in one place for convenient reference. CARRIED.

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THE CHAIRMAN said that copies of the proceedings are sent to the deans of all Canadian dental schools but that he thought it would be useful if the Secretary were to send a note to each of them

drawing attention to this feature of the proceedings.

DR. GARVIN expressed regret that Mr. Christie had left the Ontario Research Foundation where he had been working on adhesive qualities of acrylics. He stated that one manufacturer had claimed that Mr. Christie's findings as reported to the Associate Committee on Dental Research, were not based on current dental practice. In view of this observation, Dr. Garvin suggested the following as a topic for inclusion in the list of suggested research projects: To test adhesive qualities of acrylic filling material to tooth structure.

THE CHAIRMAN said this item would be added.

25. Appreciation of Catering

THE CHAIRMAN, speaking on behalf of the members, asked the Secretary to send Mrs. Karson, Manager of the Cafeteria, an expression of their sincere appreciation of the excellent luncheon which had been served to the Committee during this meeting. AGREED.

26. Date of Next Meeting

It was tentatively AGREED that the next meeting of the Committee should be held Monday and Tuesday, 2-3 March, 1953.

Meeting adjourned at 5 p.m.

APPENDIX "A"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

PAPERS PUBLISHED

Committee Paper No.	Title and Author	Reference
1	New aspects of periodontal research, by H.K. BOX	Presented at National Convention of Canadian Dentists, Toronto, 29 May, 1946. (App. "C", 5th Mtg. A.C.D.R., 25 Feb., 1947).
2	Concerning the pathogenesis of periodontal disease, by H.K. BOX	J. Periodontology, 19: 11-20. 1948. (App. "G", 7th Mtg. A.C.D.R., 2 Mar., 1948).
3	The effect of various inorganic salts on the solubility of calcium phosphate, tooth enamel and whole teeth in lactic acid, by J.J. RAE and C.T. CLEGG	J. Dent. Res. 27: 52-53. 1948.
4	Changes in the calcium and phosphate concentrations of saliva and inorganic salt solutions on shaking with calcium phosphate, by J.J. RAE and C.T. CLEGG	J. Dent. Res. 27: 54-57. 1948.
5	An improved method for processing acrylic dentures, by D.R. CHRISTIE	J. Can. Dent. Assoc., 14: 303-317. 1948. (App. "R", 8th Mtg., A.C.D.R., 26 Oct., 1948).
6	The therapeutic properties of periodontal cement packs, by W.J. LINGHORNE and D.C. O'CONNELL	J. Can. Dent. Assoc., 15: 199-205. 1949.
7	Phosphatase in saliva, by J.T. DENTAY and J.J. RAE	J. Dent. Res., Feb. 1949. 4pp.
8	The treatment of acute oral Vincent's infection, by W.J. LINGHORNE and D.F. STEDMAN	J. Can. Dent. Assoc., July, 1949. 6pp.

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10	New methods of repairing acrylic dentures without distortion, by D.R. CHRISTIE	J. Can. Dent. Assoc., 15: 582-590, 1949.
11	The corrosion of cobalt chromium alloys in commercial cleaning agents, by D.E.R. COGGLES, O.J. WALKER and H.R. MACLEAN	J. Can. Dent. Assoc. 16: 75-82, 1950.
12	The relation between buffering capacity, ciscosity and lactobacillus count of saliva, by J.J. RAE and C.T. CLEGG	J. Dent. Res. 28: 589-593. 1949.
13	Mineralization of the growing tooth as shown by Radiophosphorus autographs (17692), by L.F. BELANGER and C.P. LEBLOND	Proc. Soc. Expt. Biol. Med. 73: 390-391. 1950.
14	Radio-autographic visualization of bone formation in the rat, by C.P. LEBLOND, G.W. WILKINSON, L.F. BELANGER and J. ROBICHON	Am. J. Anat. 86: 2. 1950.
15	Fluoride studies related to the human diet, by MARY P. HAM and M. DOREEN SMITH	Can. J. Research, F, 28: 227-233, 1950.
16	Studies in the regeneration and reattachment of supporting structures of the teeth. I. Soft tissue reattachment, by W.J. LINGHORNE and D.C. O'CONNELL	J. Dent. Res. 29: 419-428. 1950.
17	An examination of the fluoride content of teeth from some Ontario districts, by SHIRLEY R. HARNESS and M. DOREEN SMITH	J. Can. Dent. Assoc., Jan., 1951.

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19	Relining acrylic dentures without distortion, by D.R. CHRISTIE	J. Can. Dent. Assoc., July, 1951.
20	Acrylic fillings - General comments and some experimental data, by D.R. CHRISTIE	J. Can. Dent. Assoc., 17: 427-435. 1951.
21	Ammonia production in saliva, by R.M. BALLANTYNE, C.T. CLEGG, J.J. RAE, and F.H. LAWFORD	J. Dent. Res. 30: 385-387. 1951.
22	Studies in the regeneration and reattachment of supporting structures of the teeth. II. Regeneration of alveolar process, by W.J. LINGHORNE, and D.C O'CONNELL	J. Dent. Res. 30: 604-614. 1951.
23	Radioactive isotopes in dental research, by J.P. LUSSIER A.D'IORIO	The Brit. Dent. Annual, 1952.

The Secretary of the Committee has a small stock of most of the papers listed and will supply copies to members of the Committee on request.

September, 1952.

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September, 1952.

APPENDIX "B"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1952

Subject: Electro potentials between dental alloys

Grantee: Dr. S.G. Davis and Dr. H.R. MacLean

Project No. 43 Amount of Grant: $150

SUMMARY OF WORK

The literature concerning the electromotive forces and currents existing between dental alloys has been reviewed. The possibility of an "in vivo" investigation as reported by Schriever and Diamond (Journal of Dental Research, 31, 205, 1952), or "in vitro" investigation is being considered. "In vitro" work has the advantage that reproducible results are obtainable and the variables can be more readily controlled and for this reason seems to warrant further investigation and it is along this line that the work will proceed. We hope this session to have a graduate student do some work on this project.

September 8, 1952.

APPENDIX "B"

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September 8, 1952.

APPENDIX "C"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1952

Subject: Oral pathology and bacteriology

Grantee: Dr. Gerald Shklar

Project No. Fellowship Grant Amount of Grant: $1500

SUMMARY OF WORK

1. THE PERIODONTIUM AND OTHER ORAL TISSUES IN THE "ALARM REACTION"

The Alarm Reaction of the General Adaptation Syndrome was studied in the albino rat by using repeated subcutaneous injections of formaldehyde as the stressor mechanism. The presence of the Alarm Reaction was demonstrated by gross and microscopic changes in the adrenal glands, thymus, spleen, gastrointestinal tract, and salivary glands. No notable clinical or microscopic changes were observed in the periodontal tissues with methods of fixation and staining routinely used in the preparation of tissues for microscopic study. Summary attached.

2. BACTERIAL ALLERGY AS A FACTOR IN THE ETIOLOGY OF GINGIVAL DISEASE. A PROGRESS REPORT

The role of oral bacteria in the etiology of gingival disease was studied with particular reference to the problem of bacterial allergy. Various tests for allergic phenomena were carried out on the guinea pig. The results of these experiments are currently being evaluated. Progress report attached.

3. THE EFFECT OF ADRENALECTOMY ON THE PERIODONTAL STRUCTURES OF THE ALBINO RAT. A PROGRESS REPORT

The periodontium was studied in the albino rat from two to eight weeks after adrenalectomy was performed. Results are currently being evaluated. Progress report attached.

September 5, 1952.

APPENDIX "C"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1952

Subject: Oral pathology and bacteriology

Grantee: Dr. Gerald Shklar

Project No. Fellowship Grant Amount of Grant: $1500

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September 5, 1952.

APPENDIX "C"

Report to the Associate Committee on Dental Research

September, 1952

1. THE PERIODONTIUM AND OTHER ORAL TISSUES IN THE ALARM REACTION[x]

by Gerald Shklar

The recent development by Dr. Hans Selye of the concept of the General Adaptation Syndrome and the Diseases of Adaptation has introduced a new viewpoint regarding the nature of disease processes. According to this concept, the reaction of the body to a wide variety of damaging influences or stress producing agents such as trauma, cold, muscular fatigue, drug intoxications, and nervous stimuli, consists of a composite of nervous and endocrinologic factors which in turn produce a syndrome of somatic changes including enlargement of the adrenal cortex, involution of lymphatic tissue, and gastrointestinal ulceration. The General Adaptation Syndrome evolves in three stages; the Alarm Reaction, the Stage of Resistance, and the Stage of Exhaustion. The Alarm Reaction is brought about by sudden exposure to stimuli to which the body is not adequately adapted. Following this stage, adaptation is acquired, but will eventually be exhausted if the damaging stimulus continues.

Of the wide variety of morphologic changes reported in the different phases of the General Adaptation Syndrome, the most interesting from the periodontal viewpoint was the "Osteoporosis of the Alarm Reaction". The fact that alveolar bone has been shown to exhibit changes similar to those seen in the remainder of the skeletal system, raised the possibility that the osteoporosis induced by stress might be a factor in the problem of chronic destructive periodontal disease. The fact that adrenocorticotropic hormone and cortisone, which play roles in the development of the General Adaptation Syndrome, have been shown to induce osteoporosis in experimental animals, is also of interest.

An experiment was thus undertaken to determine the effect of the Alarm Reaction upon the tissues of the periodontium, and the possibility of a relationship between changes in the periodontium in the Alarm Reaction and chronic destructive periodontal disease. Thirty female albino rats, Wistar strain, were divided into two groups. Group A received four subcutaneous injections of 4% formaldehyde in the groin during 48 hours, while food was withheld. Group B received six injections of the drug during 12 days. Each injection was 5 mg. Litter mates were used as control animals.

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[x]Summary of a thesis submitted in partial fulfilment of the requirements for the degree of Master of Science, Tufts College, May, 1952.

Report to the Associate Committee on Dental Research

September, 1952

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Adrenals, thymus, spleen, salivary glands, femur, and periodontium were studied microscopically. Changes seen in the adrenal gland in the Alarm Reaction were cortical enlargement, hypertrophy, and disappearance of lipid granules. Thymus and spleen demonstrated involutionary changes, consisting of necrosis of lymphocytes. Sublingual gland demonstrated a reduction in the amount of basophilic material at the base of secretory cells, disappearance of cytoplasmic granules, and some disruption of acinar architecture. In contrast to these changes, the periodontium did not exhibit notable changes, and osteoporosis was not seen in the long bones or in alveolar bone.

If the periodontal tissues were altered in this experiment, the changes were of such a nature that they could not be detected by the hematoxylin-eosin or Mallory stains. The study suggests that the Alarm Reaction, or early reaction to nonspecific stress, is not a significant factor in the initiation of chronic destructive periodontal disease. Possibly, changes could occur in the Exhaustion Stage of the General Adaptation Syndrome. The type of stressor employed in the experiment precluded an experimental period of sufficient duration to result in the Exhaustion Stage.

2. BACTERIAL ALLERGY AS A FACTOR IN THE ETIOLOGY OF GINGIVAL DISEASE. A PROGRESS REPORT

by Gerald Shklar

Background

The exact role of oral bacteria in the etiology of inflammatory disease of the gingiva has been in the subject of numerous and exhaustive studies. Bacterial allergy has been one of several factors suggested as the mechanism whereby bacteria may elicit inflammatory changes in the gingiva. Because the gingivae as well as the remainder of the oral mucosa are in constant contact with the oral bacteria and their metabolic products, it has been hypothesized that these tissues may become sensitized so that further contact with the same bacteria would be productive of an exaggerated inflammatory response. Skin sensitivity to oral bacteria has been demonstrated experimentally, and allergic cutaneous reactions have been produced with Staphylococcus aureus, Neisseria catarrhalis, Streptococcus viridans and Hemophilus influenzae, all of which are common inhabitants of the oral cavity.

In view of the sparseness of definitive information regarding the relationship of oral bacteria to the initiation and progress of gingival disease, it was felt that the role of bacterial allergy in this connection warranted exploration. A series of preliminary experiments was undertaken to investigate the possibility that allergy might be a factor in the relationship of bacteria to the initiation of gingival disease.

Experimental Procedure

Forty adult guinea pigs weighing approximately 300 grams were used as experimental animals, because of their suitability for studies in allergy. None of the animals presented any clinical evidence of gingival disease. The oral flora of the guinea pig was studied. Prominent among the large variety of microorganisms isolated from the saliva and gingival sulcus of the guinea pig were hemolytic and non-hemolytic streptococci. Because streptococci have been shown to be capable of producing allergic inflammation in many tissues of the guinea pig, a strain of non hemolytic streptococci was selected as the allergen for the present study. Heat killed suspensions (75 degrees C. for 20 minutes) in sterile physiological saline of a pure culture of non hemolytic streptococci were prepared for this purpose. The animals were divided into three groups of ten animals, with the remaining ten serving as controls. The experimental groups were subjected to the following procedures:-

GROUP I Injection Test for Gingival Sensitivity

0.5 cc. of heat killed suspension of the bacteria was injected into the gingival tissue about the right mandibular incisor tooth of

2. BACTERIAL ALLERGY AS A FACTOR IN THE ETIOLOGY OF GINGIVAL DISEASE. A PROGRESS REPORT

by Gerald Shklar

Background

The exact role of oral bacteria in the etiology of inflammatory diseases of the gingiva has been the subject of numerous and exhaustive studies. Bacterial allergy has been one of several factors suggested as the mechanism whereby bacteria may elicit inflammatory changes in the gingiva. Because the gingivae as well as the remainder of the oral mucosa are in constant contact with the oral bacteria and their metabolic products, it has been hypothesized that these tissues may become sensitized so that further contact with the same bacteria would be productive of an exaggerated inflammatory response. Skin sensitivity to oral bacteria has been demonstrated experimentally, and allergic cutaneous reactions have been produced with Staphylococcus aureus, Neisseria catarrhalis, Streptococcus viridans and Hemophilus influenzae, all of which are common inhabitants of the oral cavity.

In view of the sparseness of definitive information regarding the relationship of oral bacteria to the initiation and progress of gingival disease, it was felt that the role of bacterial allergy in this connection warranted exploration. A series of preliminary experiments was undertaken to investigate the possibility that allergy might be a factor in the relationship of bacteria to the initiation of gingival disease.

Experimental Procedure

Forty adult guinea pigs weighing approximately 300 grams were used as experimental animals, because of their suitability for studies in allergy. None of the animals presented any clinical evidence of gingival disease. The oral flora of the guinea pig was studied. Prominent among the large variety of microorganisms isolated from the saliva and gingival sulcus of the guinea pig were hemolytic and non-hemolytic streptococci. Because streptococci have been shown to be capable of producing allergic inflammation in many tissues of the guinea pig, a strain of non hemolytic streptococci was selected as the allergen for the present study. Heat killed suspensions (75 degrees C. for 20 minutes) in sterile physiological saline of a pure culture of non hemolytic streptococci were prepared for this purpose. The animals were divided into three groups of ten animals, with the remaining ten serving as controls. The experimental groups were subjected to the following procedures:-

GROUP I Injection Test for Gingival Sensitivity

0.5 cc. of heat killed suspension of the bacteria was injected into the gingival tissue about the right mandibular incisor tooth of

each experimental animal. Control animals were injected in the same area with 0.5 cc. of sterile physiological saline. It was postulated that if the gingival tissues had been sensitized from previous experience with the streptococcal organisms, the inflammatory reaction to injection of bacterial protein would be of greater intensity than that which would occur in control animals injected with physiological saline.

Observations In both experimental and control animals there was immediate erythema in a small circumscribed zone approximately 2 mm. in diameter at the site of injection. This lasted for approximately 20 minutes, followed by a return to normal. No difference could be detected between the response of the experimental and control animals.

GROUP II THE ARTHUS REACTION

Four injections of 0.5 cc. each of a heat killed suspension were injected at appropriate intervals into the gingival tissues about the mandibular incisor teeth of each animal. Control animals were similarly injected in the same area with physiological saline.

In this group of animals, an attempt was made to produce the Arthus type of reaction. This is a localized anaphylactic response in a given area which increases in severity as the result of sensitivity at the site progressively induced by succeeding injections of allergen.

Observations In both the experimental and control animals, each injection resulted in localized erythema lasting for about 20 minutes followed by a return to normal. There was no notable increase in the nature of the response to succeeding injections in either the experimental or control animals.

GROUP III THE SHWARTZMAN PHENOMENON

In this group an attempt was made to produce the Shwartzman phenomenon. This consists of an inflammatory reaction to the systemic administration of an allergen, which appears in localized areas previously "prepared" or sensitized by this same or comparable allergen. Theoretically, if the gingiva were sensitized by constant exposure to oral bacteria, systemic administration of bacterial protein might be followed by a localized inflammatory response in the gingiva.

1.0 cc. of a heat killed suspension of the bacteria was injected subcutaneously into the groin of each experimental animal. Control animals were injected with physiological saline.

Observations In five of the experimental animals, the injection of allergen into the groin was followed after six hours by a localized inflammatory response in the region of the gingiva in relation to the mandibular incisor teeth. The gingiva first appeared erythematous and slightly edematous with a glazed surface. The tissue was removed surgically for microscopic study after 48 hours, at which time the discoloration of the gingiva was intensified and in two cases there were pin point hemorrhages with surface sloughing.

Remarks

These experiments are currently being continued. It is as yet not possible to evaluate or interpret these preliminary results.

3. THE EFFECT OF ADRENALECTOMY ON THE PERIODONTAL STRUCTURES OF THE ALBINO RAT. A PROGRESS REPORT

by Gerald Shklar

The rat is comparatively resistent to adrenal insufficiency so that the effects of adrenalectomy can be studied as a chronic process, provided the animals are protected against exposure to stress, and the electrolyte balance is maintained. Adrenalectomy has been found to impair tissue metabolism and to inhibit somatic growth and the normal development of bone. It was felt that the periodontium might be involved in the generalized systemic reactions accompanying adrenal insufficiency.

A group of female Albino rats, Wistar strain were used as experimental animals. The Bilateral adrenalectomy was performed in one stage through a single dorsal midline incision at the level of the kidneys. The glands were removed together with their adherent masses of fat. The animals were kept on a low protein diet and were given physiological saline by mouth in order to maintain the electrolyte balance.

The animals were sacrificed at intervals of two to eight weeks. At the time of sacrifice, the mandible and femur were removed for microscopic examination. Observation on the periodontium and long bones are in progress.

APPENDIX "D"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1952

Subject: The Effect of the Endocrines on the Teeth and Jaws.
I. The Effect of Thiouracil on the Development of Molar Teeth of Rats.
II. The Effect of Thiouracil on the Bone of the Jaws of Rats.

Grantee: Dr. K.J. Paynter.

Project No. Dr 38 Amount of Grant: $2610.

SUMMARY OF WORK

I. The work as outlined in detail in the previous report has been completed and is now being prepared for publication. The results of the study may be briefly summarized as follows:

Propyl thiouracil-induced hypothyroidism in the rat caused a gradual slowing down of each of the processes involved in tooth formation, i.e. histo-differentiation, deposition, calcification, and eruption. All were retarded, but at an even rate, so that the end result was what appeared to be a normal tooth that simply took longer to develop.

Contrary to previous reports on tooth development after thyroidectomy, there was no effect on calcification.

II. In an attempt to ascertain whether a deficiency of thyroid hormone has any further effect if deficiency is apparent at an earlier age perhaps before organization of the dental tissues has occurred, two procedures have been followed:

a. subcutaneous administration of 6-n-propyl-thiouracil as was done before but carried on beyond twenty-five days, until the time when the third molars, which are in an early lamina stage at the onset of treatment, are completed

b. administration of the drug to pregnant rats through the drinking water starting about the twelfth day of pregnancy when the dental lamina invaginates. The young will then receive the drug through the placenta, in the milk, and, after weaning, in their own drinking water.

Litters born to such mothers have been smaller in numbers and the newborn were smaller than normal. They exhibit the dwarfism, sluggishness, and the typical proportions of cretins.

Several of them developed a hyperirritability to handling as they grew older.

None of the thyroid glands studied up to now from these hypothyroid young demonstrate the hyperplasia and hypertrophy generally associated with thiouracil-induced hypothyroidism.

APPENDIX "D"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1952

Subject: The Effect of the Endocrines on the Teeth and Jaws

I. The Effect of Thiouracil on the Development of Molar Teeth of Rats.

II. The Effects of Thiouracil on the Structure of the Bone in the Jaws of Rats.

Grantee: Dr. K.J. Paynter

Project No: DR 38 Amount of Grant: $2610

I. The Effect of Thiouracil on the Development of Molar Teeth of Rats

Beginning the day after birth daily subcutaneous injections of 6-n-propyl-2-thiouracil dissolved in 0.9% saline, were given to a group of rats. Litter-mate control animals received only 0.9% saline by the same route and in the same dose.

Animals were sacrificed in pairs, a control and an experimental, at five, ten, fifteen, twenty, and twenty-five days of age.

One half the mandible of each rat was dissected out for grenz-ray study. The remainder of the head, and the pituitary and thyroid glands were prepared for histologic study.

The following observations were noted:-

1. The characteristic dwarfing associated with hypothyroidism was observed in the animals receiving thiouracil.

2. There was evidence that each of the processes associated with the formation of teeth, i.e. histo-differentiation, deposition, calcification, and eruption, are slowed down, and at the same rate.

3. There was no evidence of a deficiency in calcification, contrary to previous reports (Ziskin and Applebaum, 1941). There was no zone of interglobular dentin, nor was the predentin zone wider than normal in the hypothyroid animals.

4. Increase in density of the cancellous bone in the mandible of the thiouracil-treated animals was apparent both histologically and in the grenz-ray at about ten days of age.

5. The differences noted between the control and experimental specimens increased with age up to twenty-five days.

The work to this point is now in the process of being prepared for publication.

Ref. Ziskin, D.E. and Applebaum, E., J.D. Res. 20:21, 1941.

II. The Effects of Thiouracil on the Structure of the Bone in the Jaws of Rats.

In the preceding study all of the molar teeth in the animals treated with thiouracil had started to form at the time treatment was initiated. The earliest development stage was in the third molars. The lamina for the third molar invaginates a day or so previous to birth.

At twenty-five days, in the treated animals the crown of the third molar was not completely developed.

Therefore it was thought that it would be of value to a) Carry animals through under the same treatment to an age when third molar crowns are completed, b) Start treatment earlier by administering the drug to pregnant rats before the time when the dental lamina appears.

By these means one could ascertain whether a deficiency of thyroid hormone at a very early age, before organization of the dental tissues occurred, could affect the size, shape or quality of the teeth.

The first experiment was repeated using animals of a different strain. Several of the experimental rats were kept alive until they were thirty-five days old. Mortality rate was high, especially after weaning, despite the fact that weaning was postponed until twenty-five or thirty days.

The heads of these animals and their controls have been prepared for study in much the same manner as was done previously. The tibias of each rat have been kept for both radiographic and histologic study.

Four other pregnant rats have been given thiouracil in their drinking water starting the twelfth day of pregnancy. (The lamina for the first molar invaginates about the thirteenth day of pregnancy).

Litters born to these rats were smaller in number than litters born to normal mothers, the young weighed less than those born to normal mothers. The average number born to the thiouracil-treated rats was eight, as compared with the average of eleven born to normal animals. The average weight of the young in the treated litter was 5.6 gms. as compared with a normal of 6.5 gms.

These animals have been killed at various ages from birth to thirty-five days of age. As was to be expected, mortality rate in the thiouracil-treated group was high. The head both tibias, and the thyroid and pituitary gland from each animal have been kept and are now being prepared for study.

All of the young showed the dwarfism associated with hypothyroidism. Several of the older specimens showed a very marked irritability on being handled.

Several of the thyroid glands of these young have been sectioned and stained. None of them demonstrate the hyperplasia and hyperemia characteristic of thiouracil treatment.

September 8, 1952.

APPENDIX "E"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1952

Subject: Bacteriology of periodontal disease. Experimental fusospirochetal infection with mixtures of pure cultures.

Grantee: Dr. J.B. Macdonald

Project No.:DR 35 Amount of Grant: $3,300

SUMMARY OF WORK

Serological studies of the motile anaerobic rods of the oral cavity were undertaken. In addition electron microscope photomicrographs were obtained of representative organisms. The data presented in relation to these two subjects is as it appears in draft form for publication as a monograph. Serological studies constitute Chapter 7 of this proposed monograph and electron microscope studies constitute a section from Chapter 4 dealing with morphologic characteristics.

Sept. 8, 1952.

APPENDIX "E"

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September, 1952

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Sept. 8, 1952.

APPENDIX "E"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

CHAPTER IV

Electron microscopy:

Strains from all 3 groups were examined and photomicrographed under the electron microscope, (Plates) a procedure which emphasized the morphologic distinctions between the 3 groups as described above and, in addition yielded further data.

Cells of Group I (strains JV7 and VB8) were mostly without flagella in the samples examined. Some cells however were seen to have 1 or 2 terminal flagella at one end of the cell. Detached flagella were also observed. The cells themselves exhibited a peculiar irregularity of surface contour which may perhaps have been the result of uneven shrinkage. Most of the cells seemed straight but a few were slightly curved.

In Group II, cultures of strain WT-OZ showing good motility including slow gliding progression were chosen for examination. Although several hundred cells were examined under the screen of the electron microscope, not a single flagellum was seen. Thus though these bacteria were motile they were clearly not motile by means of flagella. Most of their motility can be explained on the basis of flexibility of the cells but the gliding motion is not so easily explained. Creeping motion is found in the general Beggiatoa and Thiothrix and in the myxobacteria. It occurs on supporting surfaces and, in Beggiatoa, has been thought to result from waves of contraction (Ulrich, 1926). Knaysi (1951) noted that most investigators have attributed the creeping of myxobacteria to asymmetric excretion of slime which exerts pressure on one side of the cell. There is a suggestion in one of the photomicrographs (of a slime layer, and cells in the PSAFKE broth were surrounded by apparently adherent debris which may have been trapped in a slime layer. Nevertheless such an explanation of the gliding motility does not seem too reasonable. The motion of the cells was along the long axis only which does not fit the explanation and furthermore the rate of movement though slow (perhaps 4 or 5 micra per second) was a good deal faster than that of the myxobacteria (estimated by Knaysi, (1951), at 2 to 10 micra per minute) and would demand a rate of slime production which seems quite impossible. Nor does the hypothesis of contraction of cells as occurs in Beggiatoa seem any more likely. Absolutely no evidence of any change in contour of cells has been seen accompanying the gliding motility. Whatever the explanation for the gliding motility it remains at present unknown.

Cells of Group III strains are shown in Plates . Some shrinkage of the cytoplasm during drying is suggested by the clear outline of a semi-transparent cell wall beyond the opaque cytoplasm. The flagella are peritrichous. As pointed out by Knaysi (1951) it is not possible to tell by electron microscopy whether flagella originate in the cytoplasm, the cell wall or a slime layer. Due to the depth of focus of the electron microscope a flagellum appearing to penetrate the outline of the cell wall, as some do in Plates , may actually be attached to the upper or lower cell surface.

September 8, 1952.

APPENDIX "B"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

CHAPTER IV

Electron microscopy:

Strains from all 3 groups were examined and photomicrographed under the electron microscope, (Plates) a procedure which emphasized the morphologic distinctions between the 3 groups as described above and, in addition yielded further data.

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September 8, 1952.

APPENDIX "E"

Report to the Associate Committee on Dental Research

September, 1952

CHAPTER VII

SEROLOGICAL STUDIES

The most difficult investigation in the study of the motile anaerobic rods was that dealing with the serological behaviour of these organisms. This was due primarily to the very poor growth of group I strains. The poor growth created difficulties throughout most of this investigation but in the case of the serology it was the limiting factor which dictated the methods to be used. Growth was so light that when 10 ml. of a culture were centrifuged there would often be so few cells sedimented that they could not be seen. Many attempts were made to overcome this problem as indicated in Chapter V and in the following section but since they were unsuccessful it was decided to use agglutination as the means of demonstrating antigen-antibody reactions. This technique required fewer organisms than others.

The possibility of applying an immobilization technique was considered and dismissed after careful examination of many cultures under the dark-field. Cultures of group I usually had no more than 10 organisms per oil immersion field and very frequently none were motile. At best only a small percentage of organisms were motile. It seemed therefore that assessment of any difference in motility as being the result of mixture of organisms and antiserum would not be practical.

Preparation of vaccines and antigens:

Group I strains were grown in PSAFKE broth in Florence flasks filled into the neck with medium, in large test tubes (35 ml.) or in ordinary test tubes (8 ml.). Cultures were incubated anaerobically for 5 days. Many times there was no growth. In order to save medium it was found best to use ordinary test tubes and choose for centrifuging only those that showed the best growth. The recovery was so poor that it was decided to inoculate rabbits with 2 strains only - JVI and VB11A. To obtain enough organisms for preparation of vaccine and antigen for each strain required over 500 ml. of medium and over 50 tubes in which growth was obtained. At least as much medium was used in which growth failed.

Attempts were made to recover cells from growth on blood agar but the colonies seemed to grow into the agar and could not be harvested by scraping the surface. To overcome this difficulty sterile cellophane was spread over the blood agar and the inoculum

streaked on the cellophane. It was shown by Heatley (1947) that a number of organisms grew in this way. Unfortunately in this experiment no growth was obtained. Blood agar slants in screw-cap bottles were inoculated heavily and incubated anaerobically. When opened sterile saline was added to the slant, the cap replaced, and the bottle gently agitated in an effort to suspend the organisms in the saline. Gentle agitation did not release the cells. More severe agitation broke up the medium.

Group II strains were grown successfully in PSAFKE broth in 125 ml. Florence flasks or in large test tubes, incubated anaerobically for 3 days.

Group III strains were grown mostly in BX broth because this was the only medium in which growth was heavy and reliable. Antigens of these strains however were grown too in flasks of Difco N.I.H. thioglycollate broth with 30% ascitic fluid added. The BX broth contains agar. To get rid of the agar after incubation, cultures were centrifuged lightly, resuspended in saline, emulsified in a tissue grinder to release the organisms from the agar particles and filtered through glass wool. Careful examination of the filtrate showed well-dispersed cells and no agar clumps as found in the original broth. Agglutination tests using antigens prepared from BX broth and from agar-free N.I.H. broth indicated that no demonstrable antibody was being formed against any residual agar.

With cells of groups I and II addition of 0.85% saline after centrifuging usually resulted in uniform suspensions but after repeated washings suspensions often became granular. Spontaneous agglutination has been a problem with other anaerobic bacteria, particularly Fusobacterium (Böe, 1941). After considerable experimentation it was found that agglutination could be prevented at this stage by the addition of 0.01% "Tween 20" to the saline. This procedure was used routinely for preparation of all vaccines and antigens.

The following methods were used in the preparation of vaccines and antigens. Cultures were centrifuged and washed in 0.85% saline with 0.01% "Tween 20" until biuret negative. This required from 3 to 6 washings. They were then suspended in saline to a turbidity equal to tube no. 5 on the McFarland nephalometer (1907). Vaccine was prepared by heating the saline suspensions to 60°C. for 45 minutes, adding 0.5% phenol and storing in stoppered tubes. Since these organisms were motile both H and O antignes were prepared as recommended by Boyd (1942). H-antigens were prepared by addition of 0.2% formalin to the saline suspension. For preparation of O-antigen cells were centrifuged resuspended in 1 ml. of saline with 0.01% "Tween 20" to which was added 1 m. of ethyl alcohol. The mixture was incubated overnight at 37°C., held at room temperature for 3 hours and its volume then doubled with saline. Before use it was diluted further to a nephalometer reading of no. 5. O-antigens were prepared from all 5 strains of group II, both strains of group III and 4 strains of group I (JV1, VB11A, JV4, and VB8). H-antigens were prepared from the same strains with the exceptions of 2 strains from group II (WO-2 and ST-2).

Rabbits were injected intravenously with vaccines for the preparation of antisera. All rabbits were previously bled to obtain normal serum controls. Two rabbits were inoculated with each strain on 3 consecutive days each week for 3 weeks. The amounts were as follows:-

First week	- first day	0.1 ml.
	second day	0.2 ml.
	third day	0.5 ml.
Second week	- first day	0.2 ml.
	second day	0.5 ml.
	third day	1.0 ml.
Third week	- first day	0.5 ml.
	second day	1.0 ml.
	third day	2.0 ml.

Ten days after the last injection the animals were bled and the serum preserved with 1% by volume of a 1% solution of merthiolate.

Antisera were thus obtained against 2 strains of group I, (JV1 and VB11A), 3 strains of group II (Wt-OZ, B8-3 and MU-3), and 2 strains of group III (VB4 and VB10).

Agglutination experiments:

Antisera were diluted serially with 0.85% saline starting with a 1:10 dilution. In order to conserve antigen all agglutinations were carried out in small tubes (inside diameter approximately 4 mm.). Using a tuberculin syringe serum dilutions (prepared in larger tubes) were transferred in 0.1 ml. amounts to the small tubes. Each tube then received 0.1 ml. of the appropriate antigen. Ten dilutions of serum were used. In addition a saline control and a normal serum control were set up for each antigen and each antiserum.

Results:

The agglutination reactions in group I using O-antigen (Table XII) showed a moderate titre (1:320) of antibody against strain JV1 and a low titre (1:40) of VB11A antiserum against its homologous antigen. Cross-reactions occurred between JV1 antiserum and all 3 strains of group I. With the low titre of agglutination using VB11A antiserum, the absence of cross-reactions with other strains was inconclusive.

An attempt was made to raise the antibody titre of the rabbit inoculated with VB11A vaccine. Of the 2 rabbits inoculated with this strain, one died during the course of inoculations. The second rabbit was given a supplementary intravenous inoculation of 4 ml. of vaccine. Although the injection was given slowly the animal showed signs of shock within a few minutes. Breathing was very rapid and there was weakness of the hindquarters. The animal died the day following injection.

Antisera against group I strains agglutinated their homologous H-antigen only (Table XII). The titre for VB11A was 1:2560. This titre from the same rabbit which produced the low titre of antibody against the O-antigen suggests that the animal was not simply a poor antibody producer but that the somatic antigen was not strongly antigenic. The low titre against JV1 H-antigen (1:160) may have been due to the small percentage of motile cells in the cultures and hence the small number of flagella. It is likely that the amount of flagellar antigen was too small to stimulate the production of a high titre antiserum.

The results of group II agglutinations using antigens prepared with ethyl alcohol are shown in Table XIII. It will be seen that these organisms appeared by this test to be serologically unrelated except for a single cross-reaction between WT-OZ antiserum and B8-3 antigen. It should be noted that when this agglutination was performed at 50°C. instead of 37°C. the results were similar to those in Table XIII using B8-3 or MU-3 antisera but that no agglutination could be demonstrated with WT-OZ antiserum, even with its homologous antigen.

Formalinized antigens were prepared and agglutination experiments performed with group II strains before it was discovered that there were no flagella on these organisms. The antiserum titres which were recorded were almost identical with those found using antigens prepared with ethyl alcohol (Table XIII). This was further confirmation of the absence of flagella on these organisms.

The titres obtained with group III strains are shown in Table XIV. The titres were good in all cases and showed cross-reactions between strains VB4 and VB10 using both somatic and flagellar antigens. It was found possible to completely absorb the agglutinating activity of VB4 and VB10 antisera with either VB4 or VB10 O-antigen, indicating homogeneity of the O-antigens in both strains. Distinct differences in the titres using flagellar antigens occurred and provided the only definite means of distinguishing the 2 strains of group III.

Antisera and somatic antigens from all 3 groups were incubated with each other to determine whether or not cross-reactions occurred between strains of different groups. As shown in Table XV there were no cross-reactions occurred between members of different groups.

Discussion:

The small number of strains for which antisera were available made it necessary tc interpret the results with caution. These experiments lent further support however to the view that the 3 groups of organisms are distinct in their characteristics since there was no apparent serological relationship between members of different groups. Within group I and group III there were definite relationships shown between strains. The data suggests that a serological classification of groups I and III might be possible using somatic antigens to determine group and flagellar antigens to

determine strain. Such a classification is at present impractical due to the technical difficulties associated with obtaining antisera and antigens in the case of group I and obtaining enough strains in the case of group III. Furthermore, since these organisms can be easily identified by cultural characteristics, biochemical activity and morphologic criteria there would appear to be little reason to employ serological methods for this purpose unless the latter could be made simpler than the former - a contingency which at present seems quite likely.

Summary:

Rabbits were inoculated with saline suspensions of 2 strains of group I, 3 strains of group II and 2 strains of group III. Antigens were prepared from the same strains and 4 additional strains (2 in group I and 2 in group II).

Agglutination studies using somatic antigens demonstrated low titre antisera and cross-reactions amongst the 4 strains of group I. Antibodies against flagellar antigens were demonstrated. These did not cross-react with flagellar antigens of other strains of group I.

Group II strains produced low titre antisera which agglutinated the homologous antigen only. Formalinized antigens agglutinated to approximately the same titres as ethyl alcohol-treated antigens and thus produced indirect evidence of the absence of flagella on these organisms.

Somatic and flagellar antigens were demonstrated in both group III strains. Agglutinin absorption indicated that the somatic antigens were identical. The titres of flagellar antigens were not identical and provided the only means of differentiating the two strains which has been found.

TABLE XII

TITRES OF AGGLUTINATION REACTIONS IN GROUP I

ANTISERA	Somatic Antigen [1]				Flagellar Antigen [2]			
	JV1	VB11A	JV4	VB8	JV1	VB11A	JV4	VB8
JV1	320	160	40	40	160	0	0	0
VB11A	0	40	0	0	0	2560	0	0

[1] Incubated at 50°C. for 24 hours.

[2] Incubated at 37°C. for 4 hours.

TABLE XIII

TITRES OF AGGLUTINATION REACTIONS IN GROUP II

A. Antigen treated with ethyl alcohol:

Antisera	Antigens				
	B8-3	Mu-3	WT-OZ	WO-2	ST-2
B8-3	160	0	0	0	0
MU-3	0	320	0	0	0
WT-OZ	160	0	320	0	0

B. Formalinized antigen:

Antisera	Antigens		
	B8-3	MU-3	WT-OZ
B8-3	320	0	0
MU-3	0	160	0
WT-OZ	80	0	160

Incubated at 37° C. for 4 hours.
Re-examined in 24 hours.

TABLE XIII

TITRES OF AGGLUTINATION REACTIONS IN GROUP II

A. Antigen treated with ethyl alcohol:

Antisera	Antigens				
	B8-3	Mu-3	WT-O2	WO-2	ST-2
B8-3	160	0	0	0	0
Mu-3	320	0	320	0	0
WT-O2	160	0	320	0	0

B. Formalinized antigen:

Antisera	Antigens						
	B8-3	Mu-3	WT-O2				
B8-3	0	0	80	160			
Mu-3	0	160	0	0			
WT-O2	0	0	160	0	2560	40	0

1 Incubated at 50°C. for 20 hours.

2 Incubated at 37°C. for 40 hours.

Incubated at 37° C. for 4 hours.
Re-examined in 24 hours.

TABLE XIV

TITRES OF AGGLUTINATION REACTIONS IN GROUP III

ANTI-SERA	O-antigen		H-antigen	
	VB4	VB10	VB4	VB10
VB4	320	320	1280	160
VB10	1280	640	1280	2560

Incubated at 50°C. Examined in 4 hours and 24 hours.

TABLE XIV

TITRES OF AGGLUTINATION REACTIONS IN GROUP III

ANTI-SERA	O-antigen		H-antigen	
	VB4	VB10	VB4	VB10
VB4	320	320	1280	160
VB10	1280	640	1280	2560

Incubated at 50°C. Examined in 4 hours and 24 hours.

TABLE XV

TITRES IN AGGLUTINATION REACTIONS IN GROUPS I, II, AND III

A. Group I Antisera, Groups II and III O-antigens:

Antisera	Antigens				
	Group II				Gr. III
	B8-3	MU-3	WO-2	ST-2	VB10
JV1	0	0	0	0	0
VB11A	0	0	0	0	0

B. Group II Antisera, Groups I and III O-antigens:

Antisera	Antigens		
	Group I		Group III
	JVI	VB11A	VB10
B8-3	0	0	0
MU-3	0	0	0
WT-OZ	0	0	0

C. Group III Antiserum, Groups I and II O-antigens:

Antiserum	Antigens				
	Group I		Group II		
	JVI	VB11A	B8-3	Mu-3	WT-OZ
VB10	0	0	0	0	0

TABLE XV

TITRES IN AGGLUTINATION REACTIONS IN GROUPS I, II, AND III

A. Group I Antisera, Group II and III O-antigens:

Antisera	Antigens				
	Group II				Gr. III
	BS-3	MU-3	WO-2	ST-2	VBIO
JVI	0	0	0	0	0
VBILA	0	0	0	0	0

B. Group II Antisera, Groups I and III O-antigens:

Antisera	Antigens		
	Group I		Group III
	JVI	VBILA	VBIO
BS-3	0	0	0
MU-3	0	0	0
WT-O2	0	0	0

C. Group III Antiserum, Groups I and II O-antigens:

Antiserum	Antigens				
	Group I		Group II		
	JVI	VBILA	BS-3	Mu-3	WT-O2
VBIO	0	0	0	0	0

APPENDIX "F"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1952

Subject: Chemical mechanisms in dental caries

Grantee: Dr. J.J. Rae

Project No. DR 1 Amount of Grant: $1530

SUMMARY OF WORK

(1) The inhibition of ammonia production in saliva by glucose is probably due to the lowering of the pH by the acid produced by the action of the bacteria in the saliva on the glucose.

(2) Lactic acid production by saliva and the l.b.a. counts seem to be unrelated. This can be explained by the presence in saliva of an appreciable amount of acid producing bacteria other than l.b.a.

(3) The bactericidal material obtained by treating saliva with bromoform has not yet been identified although work is continuing on this project.

8 September, 1952.

APPENDIX "F"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1952

Subject: Chemical mechanisms in dental caries

Grantee: Dr. J.J. Rae

Project No. DR I Amount of Grant: $1550

SUMMARY OF WORK

(1) The inhibition of ammonia production in saliva by glucose is probably due to the lowering of the pH by the acid produced by the action of the bacteria in the saliva on the glucose.

(2) Lactic acid production by saliva and the L.b.a. counts seem to be unrelated. This can be explained by the presence in saliva of an appreciable amount of acid producing bacteria other than L.b.a.

(3) The bacteriodal material obtained by treating saliva with bromoform has not yet been identified although work is continuing on this project.

6 September, 1952.

APPENDIX "F"

ANNUAL REPORT

Interim Report No. 14

Period Covered: Feb. 1st, 1952 to Sept. 1st, 1952.

Subject: Chemical mechanisms in dental caries

Grantee: Dr. J.J. Rae

Assistant C.T. Clegg Project No. DR 1

1. Ammonia Production in Saliva

The article referred to in our last report was published in the Journal of Dental Research, Volume 31, 281, 1952. Its title was "Ammonia Production and Urease Activity in Saliva" and the authors:- Ballantyne, Rae and Lawford.

Two questions which we would like to settle arising from this work were,

(1) Why does glucose inhibit ammonia production from saliva?

(2) Why does glucose stimulate ammonia production in the presence of urea?

With regard to the first of these questions an experiment was performed in which glucose was added to buffered salivas at pH 4.0 and 8.0 in an attempt to determine if pH was the determining factor. At pH 4.0, with or without, added glucose the ammonia production in 1 hour was very small showing that a low pH (4.0) does inhibit ammonia production. At pH 8.0 the amounts of ammonia produced with or without added glucose are comparable although in two of the tubes containing glucose there was some inhibition in ammonia production and a drop in pH.

We intend to repeat this experiment with a stronger buffer at pH 8.0 but the results so far seem to indicate that pH is the controlling factor in the amount of ammonia produced. (Exp.30,33)

2. Lactic Acid Production in Saliva

It is intended to submit to the Journal of Dental Research in the near future a paper for publication on this subject. The signigicant findings as reported in our last report are,

(1) There is no relationship between l.b.a. count of saliva and lactic acid production.

(2) Even zero count l.b.a. salivas show considerable lactic acid production due no doubt to the presence of considerable numbers of other acid-producing bacteria.

3. An Inhibitory Substance for the Growth of l.b.a. in Tooth Enamel

Further work on this interesting material has shown, (1) That powdered tooth enamel must be shaken with bromoform to produce the inhibiting substance. The use of chloroform by itself produces an inactive material and the erroneous reference to its use in our previous report was based on an experiment with enamel that had had a bromoform treatment previously.

(2) The inhibiting substance produced by extraction of bromoform treated tooth enamel with acetone was found to have bactericidal action on a 48 hour culture of l.b.a. in concentrations as low as 4.7 gamma of solids per ml.

(3) In an attempt to purify this bactericidal material an experiment was performed to test its solubility in different solvents. (No. 317). 65 mg. of the inhibiting substance were dissolved in a litre of 70% methanol; the solution was put in a liquid-liquid extractor and extracted with petroleum ether (B.P. 40°-60°) for 4 hours. The ether fraction and the methanol fraction were separated and evaporated to dryness and each dissolved in 10 ml. of 95% ethanol.

The material that was contained in the methanol has a bactericidal action in concentrations as low as 0.009 mg. of solids per ml. Whereas the fraction that was contained in the petroleum ether was not inhibiting to growth in concentrations as high as 0.55 m.g. of solids per ml. The methanol fraction seemed to contain most of the inhibiting substance.

(4) The above experiment led us to the use of 70% methanol to extract the inhibiting substance instead of acetone.

This work is being continued in an attempt to identify this material and it is proposed to find out if the ash of tooth enamel produces an active material when treated with bromoform.

APPENDIX "G"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1952.

Subject: Demineralization of hard tissues as bone and teeth by organic chelating agents.

Grantee: Dr. G. Nikiforuk

Project No: DR 36 Amount of Grant: $1600

SUMMARY OF WORK

The first part of this project, dealing with methods of demineralizing hard tissues at neutral pH's, has been completed. The technique employed and the properties of the demineralizing agent (ethylene-diamine tetraacetate) are described in the enclosed report. This report will be submitted for publication, to the Journal of Dental Research, in the near future.

The second phase of the project dealing with histological studies of hard tissues prepared by demineralizing with ethylene-diamine tetraacetate is in progress. Dr. H.A. Hunter is co-operating in preparing the microscopic sections. Considerable difficulty has been encountered in preparing suitable paraffin sections. Celloidin embedding has been substituted for paraffin, with better results.

September 9th, 1952.

APPENDIX "D"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1952.

Subject: Demineralization of hard tissues as done and teeth by organic chelating agents.

Grantee: Dr. G. Nikiforuk

Project No: DR 36 Amount of Grant: $1600

SUMMARY OF WORK

The first part of this project, dealing with methods of demineralizing hard tissues at neutral pH's, has been completed. The technique employed and the properties of the demineralizing agent (ethylene-diamine tetraacetate) are described in the enclosed report. This report will be submitted for publication, to the Journal of Dental Research, in the near future.

The second phase of the project dealing with histological studies of hard tissues prepared by demineralizing with ethylene-diamine tetraacetate is in progress. Dr. H.A. Hunter is co-operating in preparing the microscopic sections. Considerable difficulty has been encountered in preparing suitable paraffin sections. Celloidin embedding has been substituted for paraffin, with better results.

September 9th, 1952.

APPENDIX "G"

Report to the Associate Committee on Dental Research

September, 1952

Subject: Demineralization of hard tissues as bone and teeth by organic chelating agents.

Grantee: Dr. G. Nikiforuk

Project No. DR 36 Amount of Grant: $1600

DEMINERALIZATION OF HARD TISSUES BY ORGANIC CHELATING AGENTS AT NEUTRAL pH'S

Gordon Nikiforuk, D.D.S., M.S.*, and Leo Sreebny, D.D.S., M.S.**

Numerous methods have been introduced to decalcify hard tissues for purposes of histological and histochemical studies. These methods have been reviewed by Ham and Harris[2] and may be listed as follows:-

1) Decalcification with weak solutions of strong acids as nitric or hydrochloric acid.

2) Decalcification with strong acids plus phloroglucin.

3) The use of ordinary fixing agents as Bouin's fluid or formic acid which also serve as decalcifying agents.

4) The use of neutral salts of magnesium citrate.

5) The acid-extraction method using 13 per cent sodium citrate solution in 45 per cent formic acid as described by Morse[4].

6) Electrolytic method.

7) More recently, Dotti[1] et al have employed an ion exchange resin and formic acid with satisfactory

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**By invitation, from the Division of Oral Pathology, College of Dentistry, University of Illinois, U.S.A.

This work was supported by a grant from the National Research Council, Ottawa, Canada.

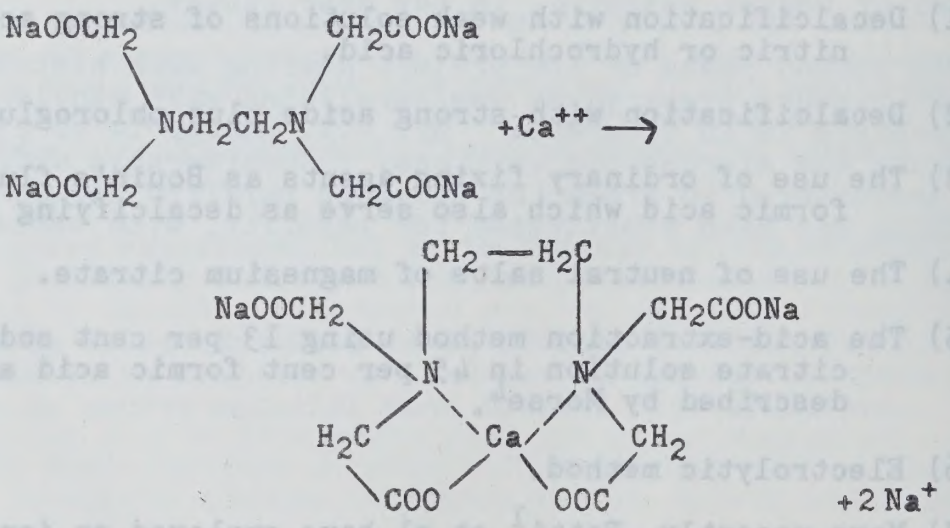
The technical assistance of Miss Sybil Vail, and preparation of illustrations by Mr. G.H. Loft is acknowledged.

results. The resin replaced the sodium citrate as a means of removing the calcium ions from solution. It will be observed that with the exception of the magnesium citrate method all decalcifying agents employ low pH's as a method of bringing bone salts into solution.

The recent introduction of a powerful organic chelating agent, ethylene-diamine tetraacetic acid (abbreviated in this paper as Edt), suggested a possible method of demineralizing hard tissues with alkaline agents. Preliminary reports[5,8] indicated that reasonably rapid demineralization of hard tissues with solutions of Edt was possible. This paper reviews the chemical properties of Edt and reports on experiments designed to determine the best method of using this agent for routine decalcification of hard tissues.

PROPERTIES OF ETHYLENE-DIAMINE TETRAACETIC ACID

The sodium salts of ethylene-diamine tetraacetic acid are non-colloidal organic chelating agents that resemble the inorganic polyphosphates (e.g., sodium hexametaphosphate) in their ability to form soluble nonionic chelates with a large number of metallic ions. The reaction with calcium is thought to proceed as follows:-



Experimentally it may be observed that a considerable drop in pH occurs when a neutral calcium salt such as calcium chloride is added to a solution of the disodium salt of Edt. This is due to the liberation of hydrogen ions in the course of complex formation:-

$$Ca^{++} + Na_2H_2Edt \rightleftharpoons Na_2(CaEdt) + 2H^+$$

This phenomenon makes it necessary to buffer Edt solutions when large amounts of calcium are to be chelated and where drops in pH are undesirable.

The effect of pH on the chelating power of Edt towards calcium is illustrated in Fig. 1 which is taken from Technical Bulletin No. 2 Bersworth Chemical Company. It is noted that calcium is strongly chelated above pH 6 and that the curve levels off at pH's above 7.5. The efficiency drops markedly below pH 6. This property of Edt makes valuable as a demineralizing agent.

Edt is soluble to the extent of about 0.03 per cent at room temperature. Di-sodium di-hydrogen salt is soluble to the extent of 6 per cent. The tri- and tetra-sodium salts are very soluble and dissolve to the extent of 30 and 50 per cent respectively, at room temperature.

The negative logarithm of the dissociation constants are reported by Schwarzenbach and Ackermann[7] to be 2.0, 2.67, 6.16 and 10.26 at 20°C. for the first, second, third and fourth hydrogen ion, respectively. The reader is referred to the above paper for a more complete account of the equilibria between the combining ions.

MATERIAL AND METHODS

The acid and sodium salt of Edt was obtained from the Bersworth Chemical Company, Freemingham, Mass. under the trade name of Versene. The acid salt, obtainable in 100 per cent dry powder, was neutralized to desirable pH's by using solutions of sodium hydroxide of suitable strength. The sodium salt of Edt, obtainable in 34 per cent liquid form, was adjusted to desirable pH's by the use of hydrochloric or citric acids.

Bones used to test solutions of Edt for its demineralizing property were obtained by sacrificing 120 day old littermate rats. The femur, tibia and mandible bones of these rats were stripped of all external soft tissue, fixed in 10 per cent formalin and used for decalcification studies with Edt. The individual bones differ in chemical composition and are not quantitatively comparable. However, when bones obtained from different rats, of the same litter and age, were placed in identical solutions of Edt, the demineralization rate is approximately the same. Hence, it was possible to roughly quantitate the results obtained. Each experiment was run in duplicate and the results reported were readily reproducible.

The rate of demineralization was determined by X-raying the specimens at different intervals. The absence of radio-opacity was taken as evidence of complete demineralization. The use of X-rays for assessing the rate of demineralization, though satisfactory, is only roughly quantitative.

All specimens used for demineralization, unless states otherwise, were suspended near the top of a column of 500 ml. of the demineralizing solution at room temperature.

EXPERIMENTAL FINDINGS

1) Effect of pH on the Rate of Demineralization of Bone Specimens by Edt:-

Four rat mandibles and four femurs, previously fixed in 10 per cent formalin, were suspended in 500 ml. of 0.25 M of Edt solution, 25°C. at pH's of 6.02, 7.49, 9.53 and 12.01. Table 1 summarizes the results obtained.

Table 1

Time Required for Complete Demineralization of Rat Mandibles Suspended in 500 ml. of 0.25 M.Edt at Varying pH's, 25°C.

pH	6.02	7.49	9.53	12.01
Days required for demineralization	8	9	16	18
pH after demineralization	6.03	7.49	9.50	11.77

Figures 2 (a) and (b) show roentgenograms of rat mandibles and femurs taken seven days after specimens were placed in solutions of Edt at pH's of 6.02, 7.49, 9.53, and 12.01. The bone specimens suspended in solutions of pH 6.02 and 7.49 are almost completely decalcified; the bone specimens suspended in solutions of pH 9.53 and 12.01 remain rather highly calcified. The optimum pH for demineralization of bone specimens by Edt is around the neutral point. These results do not parallel the curve in Figure 1 for reasons discussed below.

2) Effect of Concentration of Edt on the Rate of Demineralization of Bone Specimens:-

Rat mandkbles, weighing approximately 0.40 grams, were suspended in solutions of Edt, pH 7.43, at concentrations varying from 0.1 to 0.9 M. One week later the specimens were X-rayed to determine extent of demineralization - Figure 3. The length of time required for complete demineralization of bone specimens shown in Figure 3, is represented graphically in Figure 4. The experiment indicated that increasing the concentration of Edt from 0.1 M to 0.5 M results in a discernible increase in the velocity of demineralization of the bone specimens. Increasing the concentration of Edt beyond 0.5 M does not appreciably alter the speed of demineralization of small bone specimens.

3) Comparison of Rate of Demineralization of Bone Specimens by Edt Various Temperatures and with other Decalcifying Agents: -

According to Schwarzenbach and Ackermann[7] the complex of Edt and calcium is stable over a wide range of temperature. It was

important, therefore, to determine whether the rate of demineralization of bone tissue is significantly increased by elevating the temperature of the Edt solutions. Rat femurs were suspended in solutions of Edt, 0.5 M; pH 7.49, at temperatures of 4°, 25°, 37.5° and 60°C. Table 11 indicates that demineralization of bone specimens is greatly increased by elevating the temperature of the solutions. This result has important practical connotations.

A comparison of the rate of demineralization of bone specimen by various decalcifying agents (Table 11) indicates that at 25°C., solutions of Edt are approximately 1/7 as effective as a 5 per cent solution of nitric acid; however, at a temperature of 60°C. the solution of Edt decalcifies as rapidly as a 5 per cent solution of nitric acid at room temperature. Solutions of Edt decalcify bone at approximately one-half the speed required by solutions of formic acid - sodium citrate. Solutions of Edt are much more effective for demineralization of bone than neutral solutions of magnesium citrate.

Table 11

Comparison of Rate of Demineralization of Bone Specimens by Edt, ph 7.49; 0.5 M, at Various Temperatures and with other Decalcifying Solutions of Equal Volume.

Demineralizing Solution	Edt	Edt	Edt	Edt	5% HNO_3	Formic citrate	Formic resin	Mag. citrate
Temperature	4°C.	25°C.	37.5°C.	60°C.	25°C.	25°C.	25°C.	25°C.
Approximate days for complete demineralization	10	7	3	1	1	3	1	35

4) Additions of Accelerators to Edt and their effect on the rate of Demineralization:-

Hahn and Reygadas[3] have reported that the neutralization of Edt by sodium pyrophosphates instead of sodium hydroxide speeds decalcification of "iron containing tissue". Recently, Dotti[1] et al have employed exchange resins together with formic acid to increase the velocity of demineralization of tissues. Sodium citrate has been used to remove ionic calcium from solutions. These reagents were tried for their effect on velocity of demineralization of bone by Edt.

The effect of sodium pyrophosphate was investigated by preparing a solution of Edt of 0.5 M, pH 7.4 as follows:-

146 grams of Edt acid powder and 30 grams of sodium pyrophosphate were placed in a large beaker and water added to make a thick paste. The addition of strong NaOH permits the Edt to go into solution. Enough NaOH is added to obtain a pH of 7.4. Water is added to bring the volume up to one litre.

Because of its low solubility it was not possible to prepare a solution of Edt of 0.5 Molarity and pH 7.4 by neutralizing with sodium pyrophosphate alone. This solution decalcified small rat femurs approximately two days sooner than a control solution of the same molarity and pH but without sodium pyrophosphate.

The rate of demineralization of a solution of Edt neutralized with citric acid was not significantly different when compared with a solution neutralized with hydrochloric acid to a similar pH.

The addition of a resin (ammonium salt of a sulfonated resin) to Versene, at neutral pH's, did not increase the velocity of demineralization of bone specimens.

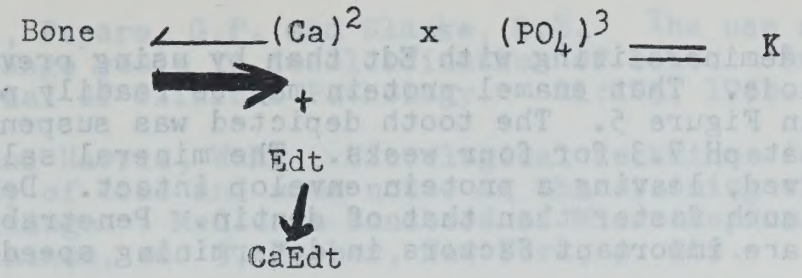
5) Effect of Suspending Bone Specimens at different Heights in a Column of Edt:-

Hahn and Reygadas[3] stated that mother of pearl buttons decalcified faster when the specimens were suspended near the top of the column of the solution. A similar experiment with bone specimens indicated that demineralization of bone specimens placed at the bottom of a vessel is much slower than demineralization of similar specimens suspended several inches from the bottom. Raising the specimen further did not appreciably increase the velocity of demineralization.

DISCUSSION

The ability of ethylene-diamine tetraacetate to form complexes with calcium salts at neutral and alkaline pH's offers a new and satisfactory method of demineralizing hard tissues without subjecting the tissue specimens to low pH's and concomittant drastic denaturation of protein.

Demineralization of hard tissues by Edt is best explained on the principle of the solubility product constant. When a difficulty soluble substance such as bone or a tooth is placed into some water, a small but very definite amount of these substances dissolves. For didactic purposes, it is assumed that bone is predominantly $Ca_3(PO_4)_2$. Then, when placed in water a definite amount of calcium and phosphate will dissolve, until the bone is in equilibrium with its saturated solution. The product of the molal concentrations of the calcium and phosphate ions must equal a constant at a given temperature. The constant denoted by the letter "K" is the solubility product constant of bone. If now Edt is added, some calcium ions are withdrawn from the solution. More bone substance will dissolve to satisfy the solubility product constant. The equilibrium is thus shifted to the right, as illustrated by the equations below, until the bone specimen is completely dissolved.



The above principle also explains the apparent discrepancy between the optimal pH of Edt for chelation of calcium and for demineralization of hard tissue. Figure 1, illustrates that Edt chelates calcium best at alkaline pH's, and that the curve flattens above pH 7.5. However, at pH's above 10.5 the rate of demineralization of bone specimens by Edt is slower than at neutral pH's. With fewer calcium ions available at a pH $>$ 10 than $<$ 10, the rate of demineralization is not as rapid.

Experimental findings (Table 1) indicate that a pH of 7-8 is suitable for demineralizing hard tissue specimens. A concentration of 0.5 of Edt is sufficient for specimens weighing 0.5 $\pm$ 0.1 grams. When 500 ml. of Edt solution is used, changing solutions daily is not necessary. The chelating capacity of Edt is so great that only a fraction of available Edt is used up during demineralization of small specimens. Thus, for small bone specimens (up to 1 gram), changing solutions is unnecessary. Experimental findings also suggest that the use of sodium pyrophosphate is valuable in increasing the velocity of demineralization of large bone specimens. Increase of temperature speeds demineralization markedly, and may be used where rapid demineralization is required. Specimens should be suspended near the top of the column of liquid. The complex-containing solution, having a higher density, sinks bringing about an automatic circulation and renovation of the reagent3.

The greatest disadvantage in using Edt, as compared with acid solutions, is that the rate of demineralization is slower. Bone specimens weighing approximately 0.4 grams may be decalcified by 5 per cent nitric acid in one day. A comparable specimen may require 2-3 days with formic-citric acid; 5-8 days with Edt at pH 7.4 at 25°C; one day with formic acid-resin; 30 days when neutral solution of magnesium citrate is used. Thus Edt solutions have approximately 1/7 the velocity of demineralization of acid solutions; on the other hand, they are about seven times as fast as any existing neutral solution used for demineralization of hard tissues.

The use of Edt for demineralization purposes should prove useful in histochemical, chemical and histological stidies os small specimens of calcified tissues. The action of Edt solutions is very mild as contrasted with acid solution. The protein fraction is not as easily destroyed and the development of CO_2bubbles is prevented. This suggests that enamel protein may be more easily preserved and

recovered by demineralizing with Edt than by using previously described methods. That enamel protein may be readily preserved is illustrated in Figure 5. The tooth depicted was suspended in a 0.5 M Edt solution at pH 7.3 for four weeks. The mineral salts of the enamel dissolved, leaving a protein envelop intact. Demineralization of enamel is much faster than that of dentin. Penetrability and surface area are important factors in determining speed of demineralization.

Preliminary experiments indicate that bone tissues demineralized by Edt possess staining properties that are vastly different than tissues decalcified by acid solutions. This result would be expected, since the affinity of tissues for various dyes is influenced considerably by the pH of the solutions used.

The histological phase of this project is now in progress and will be the subject of a future paper.

SUMMARY

(1) A new method for demineralizing calcified tissues at neutral pH's is described. The demineralizing agent is an organic chelating agent - ethylene-diamine tetraacetic acid.

(2) The Chemical properties and the experimental findings relative to the use of this agent as a decalcifying agent are reported.

(3) The acid powder neutralized to a pH of approximately 7.4 by the use of sodium hudroxide, or the sodium salt of ethylene-diamine tetraacetic acid neutralized to the same pH by hudrochloric acid, in a concentration of 0.5 M, at room temperature, makes a suitable demineralizing media for small specimens (weighing about 0.5 grams) of bone tissue.

(4) The use of higher temperatures greatly increases the speed of demineralization of bone specimens by Edt.

(5) Edt solutions are useful at a neutral pH; hence are mild demineralizing agents. This method should prove useful in histological, histochemical and chemical studies of bone tissues.

(6) Edt solutions demineralize bone specimens at a rate about 1/7 that of acid solutions and about seven times as rapidly as a neutral decalcifying solution of magnesium citrate, developed by Kramer and Shipley[2].

(7) The mechanism of action of Edt as a demineralizing agent is described.

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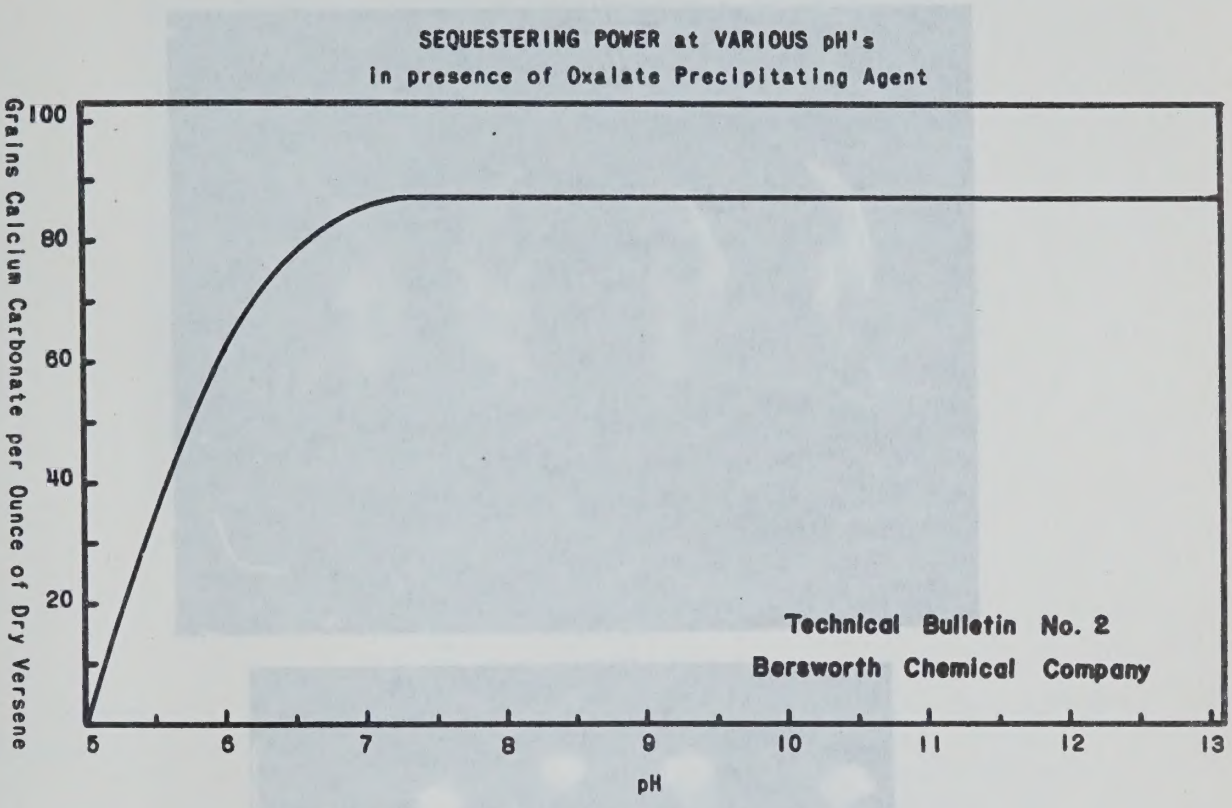
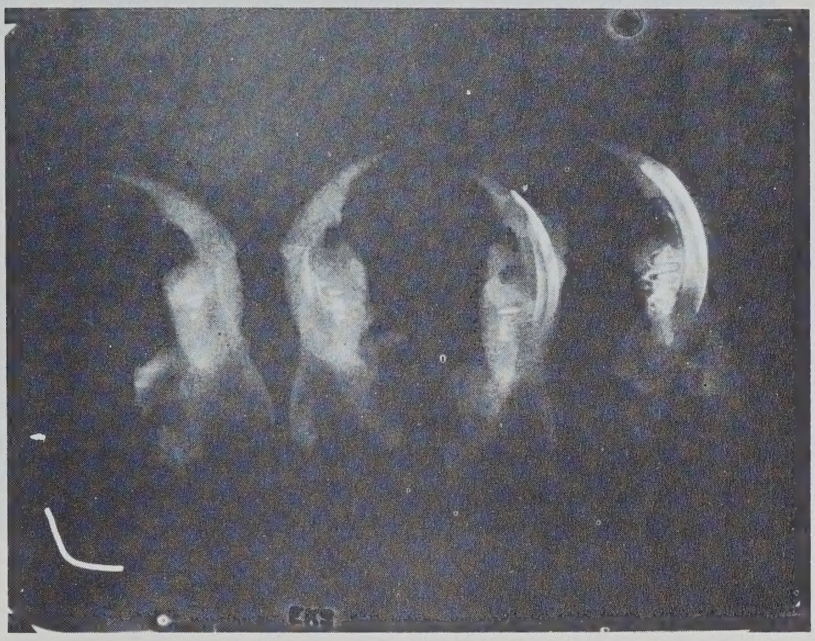
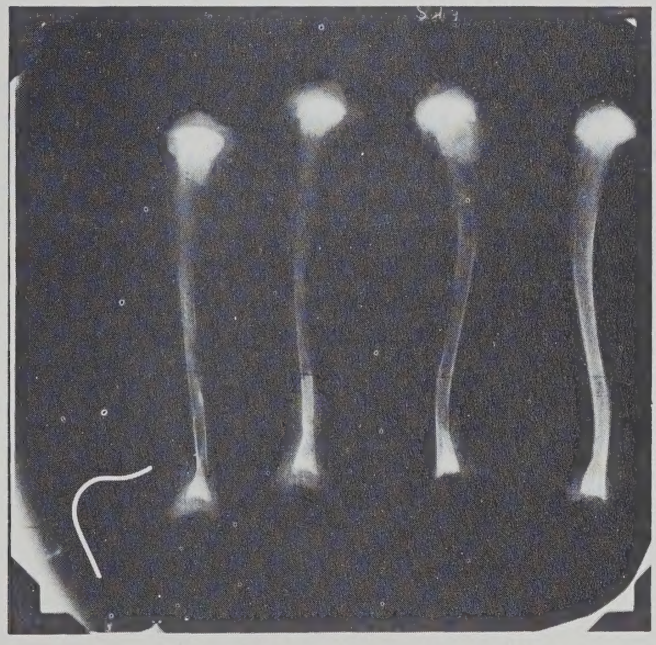


Figure 1:- Effect of pH on the chelation of calcium by ethylene-diamine tetraacetic acid.

100
80
60
40
20
5
6
7
8
9
10
11
12
13
pH





Figures 2 (a) and (b):- Roentgenograms of rat mandibles and femurs seven days after specimens were suspended in solutions of Edt at pH's 6.02, 7.49, 9.53, 12.01 from left to right, respectively.

Figure 3:- Roentgenograms of rat mandibles taken seven days after suspension in solution of Edt at pH 7.43 at room temperature. From left to right, the upper and lower radiograms represent bone specimens that were placed in 0.1, 0.2, 0.3, 0.4, 0.5, 0.7, 0.9 M solution of Edt respectively.

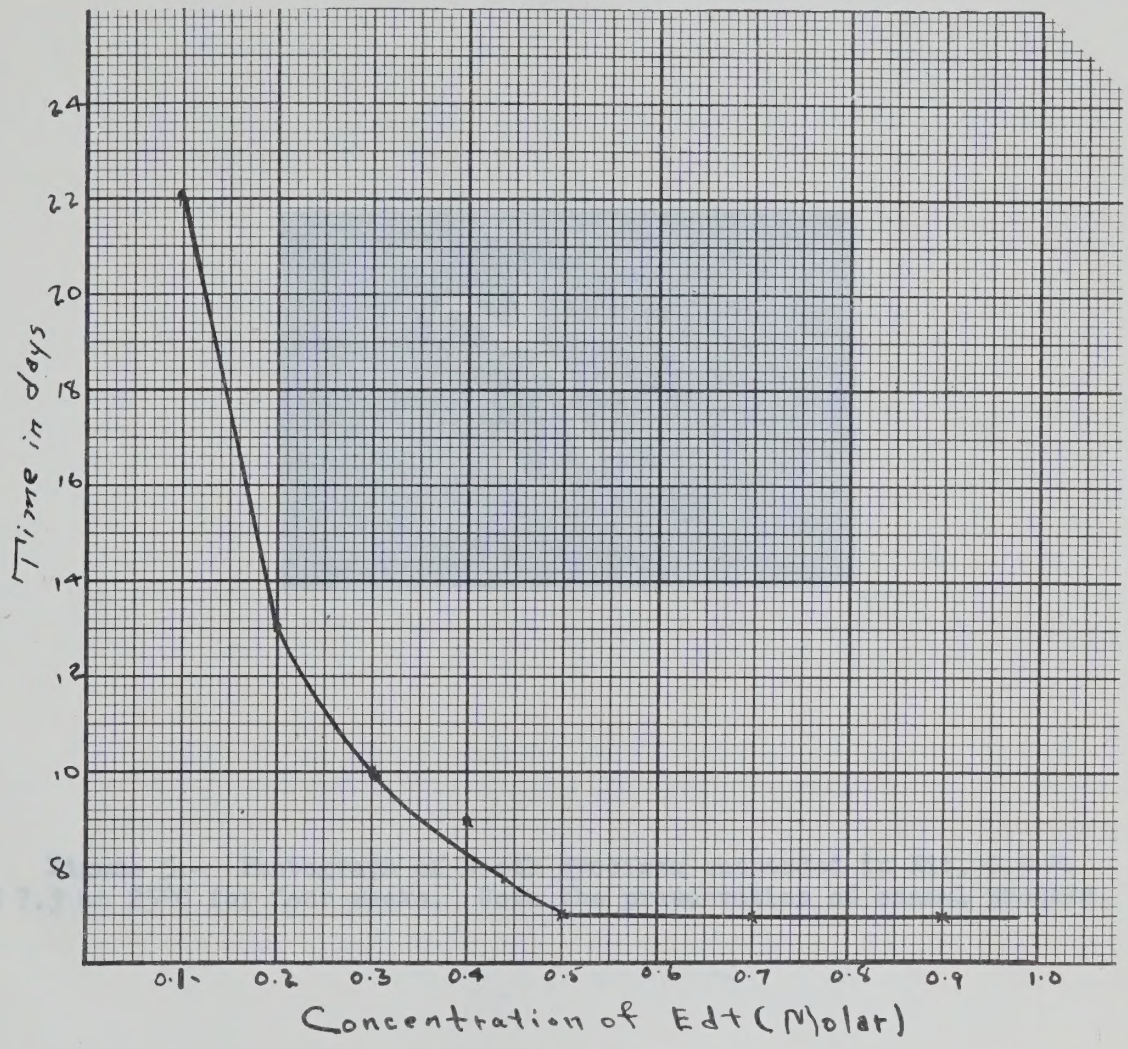


Figure 4:- Graph to indicate the effect of concentration of Edt on velocity of demineralization of bone specimens (Rat Femurs) weighing approximately 0.40 grams at pH 7.40 at room temperature.

Figure #1- Graph to indicate the effect of concentration of HCl on velocity of decalcification of bone specimens (Bat Femure) weighing approximately 0.40 grams at pH 7.40 at room temperature.

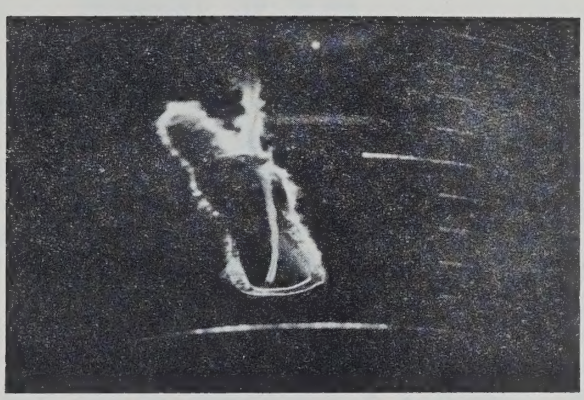


Figure 5:- Photograph of tooth specimen suspended in Edt, 0.5 M, pH 7.3 at 25°C for four weeks. Note the preservation of enamel protein.

APPENDIX "H"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1952.

Subject: Studies in the chemistry of the saliva. II. The reducing property of saliva towards some oxidation-reduction indicators.

Grantee: Dr. G. Nikiforuk

Project No. DR 42 Amount of Grant: $1500

SUMMARY OF WORK

The reducing property of saliva towards several oxidation-reduction potential indicators is being investigated. The indicators selected are those whose E^{o} values (potentials of half reduced dyes at $_{p}H$ 7.0) cover the range of most biological enzyme systems. The dyes selected are: - toluylene blue (E^{o} = +0.115v), cresyl blue (E^{o} = +0.047v), methylene blue (E^{o} = +0.011v), 2,3,5 triphenyl tetrazolium chloride (E^{o} = -0.08v), cresyl violet (E^{o} = -0.170v), and safranin (E^{o} = -0.289v). (See Figure 1). Preliminary experiments indicate that saliva readily reduces dyes whose E^{o} at $_{p}H$7.0 is more positive than -0.20v. This E^{o} corresponds to that of the lactate-pyruvate system. This is interesting in view of the fact that lactic dehydrogenase will reduce the oxidized form of all indicators whose E^{o} at $_{p}H$ 7.0 is more positive than -0.20v (e.g. cresyl violet).

The results of other experiments using methylene blue, 2,3,5-triphenyl tetrazolium chloride as indicators and saliva stimulated by paraffin chewing are as follows:-

(1) Boiling saliva for five minutes destroys its ability to reduce redox indicators.

(2) When saliva is permitted to stand for 30 minutes the activity is largely recovered in the sediment; the supernatant is largely inactive.

(3) When centrifuged, the supernatant has little activity. The sediment when suspended in 0.1M phosphate buffer, $_{p}H$7.0 contains 65 per cent of the original activity.

(4) Saliva filtered through Whatman No. 7 filter paper loses most of its activity.

(5) Saliva put through a Seitz filter loses all its activity.

The above results suggest that the ability of saliva to reduce redox indicators is associated with the presence of solids (probably bacteria and desquamated cells) in the saliva.

(6) The speed at which mixed saliva reduces redox indicators is increased by the addition of sodium lactate.

(7) The speed at which various indicators are reduced varies greatly from one individual to another (from several minutes to several days). An attempt will be made to relate the velocity of reduction with certain clinical conditions as Vincent's infection (trench mouth) and dental caries.

Professors, A.M. Wynne and G.C. Butler from the Department of Biochemistry, University of Toronto, are assisting in this project.

September 9, 1952.

APPENDIX "B"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1952.

Subject: Studies in the chemistry of the saliva. II. The reducing property of saliva toward some oxidation-reduction indicators.

Grantee: Dr. G. Mikiforuk

Project No. DR 42

Amount of Grant: $1500

SUMMARY OF WORK

The reducing property of saliva toward several oxidation-reduction potential indicators is being investigated. The indicators selected are those whose E^o values (potentials of half reduced dyes at $_pH$ 7.0) cover the range of most biological enzyme systems. The dyes selected are: - tolylene blue (E^o = +0.11Sv), cresyl blue (E^o = +0.04v), methylene blue (E^o = +0.01v), 2,3,5 triphenyl tetrazolium chloride (E^o = -0.08v), cresyl violet (E^o = -0.17Ov), and safranin (E^o= -0.289v). (See Figure 1). Preliminary experiments indicate that saliva readily reduces dyes whose E^o at $_pH$7.0 is more positive than -0.20v. This E^o corresponds to that of the lactate-pyruvate system. This is interesting in view of the fact that lactic dehydrogenase will reduce the oxidized form of all indicators whose E^o at $_pH$ 7.0 is more positive than -0.20v (e.g. cresyl violet).

The results of other experiments using methylene blue, 2,3,5-triphenyl tetrazolium chloride as indicators and saliva stimulated by paraffin chewing are as follows:-

(1) Boiling saliva for five minutes destroys its ability to reduce redox indicators.

(2) When saliva is permitted to stand for 30 minutes the activity is largely recovered in the sediment; the supernatant is largely inactive.

(3) When centrifuged, the supernatant has little activity. The sediment when suspended in 0.1M phosphate buffer, $_pH$7.0 contains 65 per cent of the original activity.

(4) Saliva filtered through Whatman No. 7 filter paper loses most of its activity.

(5) Saliva put through a Seitz filter loses all its activity.

The above results suggest that the ability of saliva to reduce redox indicators is associated with the presence of solids (probably bacteria and desquamated cells) in the saliva.

(6) The speed at which mixed saliva reduces redox indicators is increased by the addition of sodium lactate.

(7) The speed at which various indicators are reduced varies greatly from one individual to another (from several minutes to several days). An attempt will be made to relate the velocity of reduction with certain clinical conditions as Vincent's infection (trench mouth) and dental caries.

Professors, A.M. Wynne and G.C. Butler from the Department of Biochemistry, University of Toronto, are assisting in this project.

September 9, 1952.

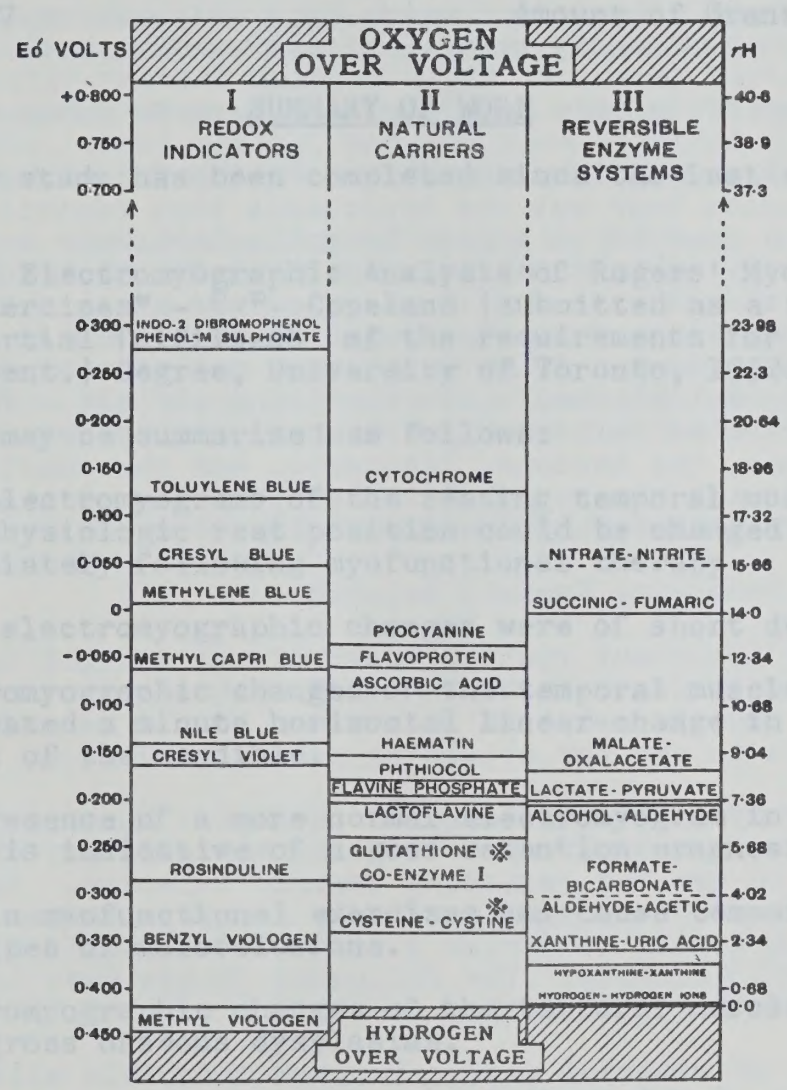


Figure 1:- The E^{o} at pH 7.0, at 30' of oxidation reduction potential indicators (From Stephanson, Bacterial Metabolism. p. 39).

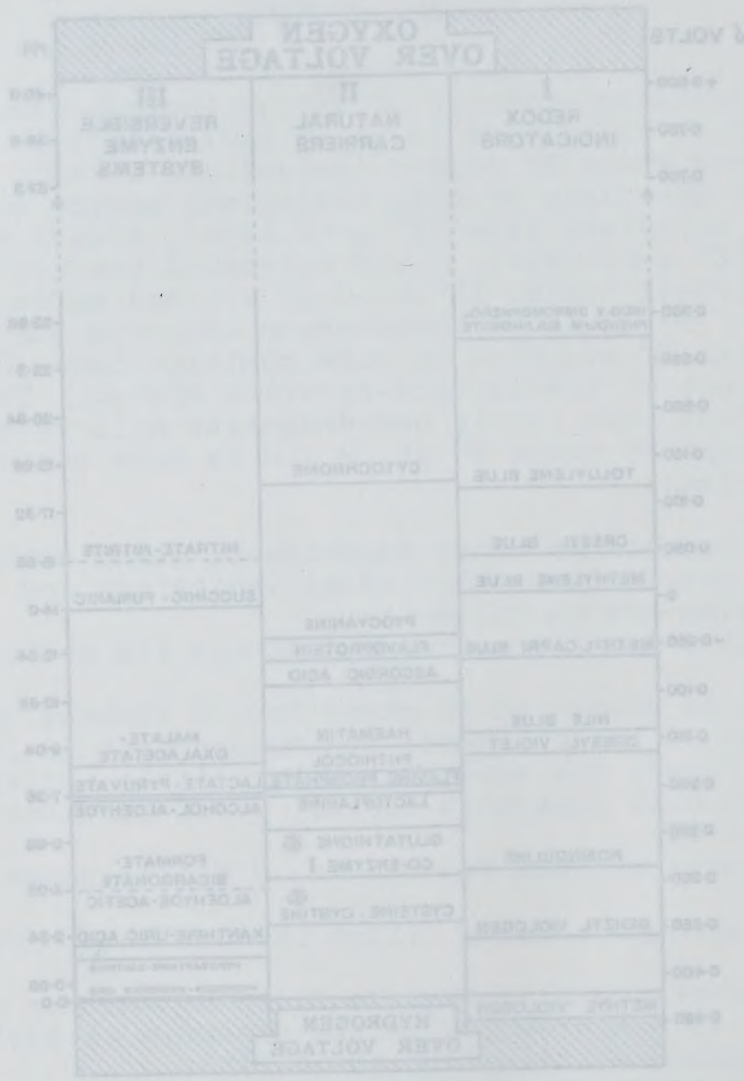


Figure 1:- The E_h^o at pH 7.0, at 30° of oxidation reduction potential indicators (From Stephenson, Bacterial Metabolism, p. 39).

APPENDIX "I"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1952

Subject: Further studies in the electromyography of the head, face and neck.

Grantee: Dr. Robert E. Moyers

Project No. DR 37 Amount of Grant: $800

SUMMARY OF WORK

I The following study has been completed since the last report on this project:

"An Electromyographic Analysis of Rogers' Myotherapeutic Exercises" - G.P. Copeland (submitted as a thesis in partial fulfilment of the requirements for the M.Sc. (Dent.) Degree, University of Toronto, 1952.

The findings may be summarized as follows:

a. Some electromyograms of the resting temporal muscles with the mandible in physiologic rest position could be changed to a more normal pattern immediately following myofunctional therapy.

b. These electromyographic changes were of short duration.

c. Electromyographic changes of the temporal muscles to a more normal pattern indicated a minute horizontal linear change in the physiological rest position of the mandible.

d. The presence of a more normal electromyogram in a treated malocclusion is indicative of a good retention prognosis.

e. Certain myofunctional exercises can cause temporal E.M.G. changes in certain types of malocclusions.

f. Electromyographic changes of the temporal muscles cannot be expected in gross osseous dysplasias.

g. Myofunctional therapy is contraindicated in most adult malocclusions and in all cases of gross osseous dysplasia.

II Three studies are in progress:

A. "An Electromyographic Analysis of Distocclusion" with R.D. Harvett. This is a development of a previous paper (1). The work thus far may be summarized as follows:

All distocclusion cases may be thought of originally as (1) skeletal, wherein there is a gross osseous dysplasia, (2) dental, wherein teeth have moved or been moved from their desired positions, or (3) muscular, wherein there is malfunction of the muscles moving the mandible. Only in very young childhood are the true typal forms seen. By adolescence most clinical problems are a combination of two or more of these etiologic types. Our experimental sample is divided into distocclusions in two age groups, (1) 5-6 years old and (2) 12 years old. Cephalometric and electromyographic analyses of each patient are being studied. An endeavour is being made to find methods of recognizing the several types of this clinical problem. There is considerable evidence already that one of the types is not ordinarily treated at the optimum age.

B. "The Physiology of Centric Relation" - Robert E. Moyers.

The electromyograph is an excellent device for studying centric relation. Our studies bear out the hypothesis that centric relation is primarily neither a condyle to eminentia articularis nor a denture to denture relationship, as is traditionally assumed. Centric relation does not exist in the neonate and is only learned after the arrival of teeth and the instigation of mastication. It is a learned neuromuscular reflex contraction pattern. As with all such neuromuscular habits, it becomes more firmly established with repetition and age. The afferent arms of this complicated reflex arise from proprioceptive organs in the periodontal membrane, the tendons, ligaments, and the muscles themselves. Centric relation is not the postural reflex position of the mandible but rather an oft repeated functional position.

C. Methods of Recording Centric Relation.

One of the important routine tasks of the dentist is that of recording centric relation. It was natural that along with the study of the physiology of centric relation an attempt should be made to analyze the various methods in use for obtaining centric relation.

The electromyographic record can show most accurately centric relation and thus serves as a way of testing the efficacy of the many methods currently in use for recording centric relation. None of the procedures tested have been found routinely accurate and we have directed some effort towards trying to devise a reliable and valid method which might be used by all dentists. The following things have been shown to affect the accuracy of the method used:

1. Consistency of material used. Tougher materials elicit a protrusive positioning of the mandible.

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(1) Moyers, R.E. Temporomandibular muscle contraction patterns in Angle Class II div. 1 malocclusions: an electromyographic analysis. Amer. Jour. Ortho. 35:837-857, 1949.

2. Weight of material placed in the mouth. Some of the techniques require that up to two pounds of face bows, trays, etc. be attached to the mandible (e.g. the McCollum gnathoscopic method). It is impossible to obtain a correct centric relation under such conditions.

3. Psychic factors:

a. Taste of material - e.g. plaster of paris dehydrates the oral mucosa and the patient reflexly tenses, destroying any hope of precision in recording.

b. Apprehension - Various drugs have been tested to note their effects. (1) Sodiumpentobarbital relieves anxieties in the patient's mind and thus indirectly relaxes muscles. (2) Tolserol works on the internuncial neurone and serves as a more direct muscle relaxant. This latter drug is non-toxic, short acting, and may have clinical possibilities. It is being supplied by one of the several manufacturers, E.R. Squibb & Sons.

c. It has also been observed that, in the same subject, a more accurate record can be obtained when the patient is very tired.

4. Contacting teeth. It is important when recording centric relation in dentulous subjects to use a material which prevents the teeth contacting one another. This minimizes any afferent impulses from premature dental contact which might inhibit, impede or alter the basic reflex contraction pattern.

Dr. Charles Moses of the Department of Prosthetics is aiding this portion of the study.

APPENDIX "J"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1952

Subject: A study of the normal and abnormal physiologic forces of the oral cavity.

Grantee: Dr. Robert E. Moyers

Project No. DR 26 Amount of Grant: nil

SUMMARY OF WORK

I Three studies have been completed, since the last time of reporting, which were carried out with the apparatus provided under this project.

1. "Forces of the Tongue against the Hard Palate incurred during Deglutition and their Relationship to the Maxillary Arch" - Donald P. Hult (submitted as a thesis for the M.Sc. (Dent.) degree, April 1952.

2. "The Selection of Forces in Orthodontic Treatment" - Dr. Holly Halderson, Dr. E.E. Johns, and Dr. Robert E. Moyers. Read before the 48th annual meeting of the American Association of Orthodontists, St. Louis, Mo., April 1952. Accepted for publication by the American Journal of Orthodontics. (This is one of three parts in a symposium given by the University of Toronto, Department of Orthodontics.)

3. "An Analysis of the Forces Inherent within the Standard Orthodontic Appliances" - Dr. E.E. Johns. (To be submitted as a thesis for the B.Sc. (Dent.) degree.

II A new study has just been started which will be reported later. Dr. Bruce Morrow is analyzing the role of lip and buccal pressures in the development of the dentition and the genesis of malocclusion.

APPENDIX "J"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1952

Subject: A study of the normal and abnormal physiologic forces of the oral cavity.

Grantee: Dr. Robert E. Moyers

Project No. DR 26 Amount of Grant: nil

SUMMARY OF WORK

I Three studies have been completed, since the last time of reporting, which were carried out with the apparatus provided under this project.

1. "Forces of the Tongue against the Hard Palate Incurred during Deglutition and their Relationship to the Maxillary Arch" - Donald P. Hult (submitted as a thesis for the M.Sc. (Dent.) degree, April 1952.

2. "The Selection of Forces in Orthodontic Treatment" - Dr. Holly Haldorson, Dr. E.E. Johns, and Dr. Robert E. Moyers. Read before the 45th annual meeting of the American Association of Orthodontists, St. Louis, Mo., April 1952. Accepted for publication by the American Journal of Orthodontics. (This is one of three parts in a symposium given by the University of Toronto, Department of Orthodontics.)

3. "An Analysis of the Forces Inherent within the Standard Orthodontic Appliances" - Dr. E.E. Johns. (To be submitted as a thesis for the B.Sc. (Dent.) degree.

II A new study has just been started which will be reported later. Dr. Bruce Morrow is analyzing the role of lip and buccal pressures in the development of the dentition and the genesis of malocclusion.

APPENDIX "K"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1952

Subject: Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radio-active elements.

Grantee: Dr. C.P. Leblond

Project No.: DR 23 Amount of Grant: $3,000

SUMMARY OF WORK

The work carried out with the help of this grant is designed to improve our knowledge of the mechanism controlling the growth of the tooth. The work done with radioactive phosphate and radioactive calcium supplies information on the mineral part of the tooth, while the work carried out with radio-carbon gives results related to the development of the organic matter. This work was done with the collaboration of Mr. R.C. Greulich, who studied the entry of radio-carbon. His report is enclosed, as well as the first draft of a paper on the subject.

A good deal of work was done in an effort to find an improved method to study teeth that have matured long enough to have become quite hard. Two different methods were used. On the one hand, Mr. M.Weiss has used a slicing machine which he has developed on the basis of an article by Atkinson. His report is enclosed as well as photographs of the machine. Another method which was attempted was that of Arnold, initially developed to make sections of mature bone. In this laboratory, Miss Yurika Kumamoto has attempted to adapt this method to work on the growing tooth. These results are enclosed in a separate report.

The main results obtained in the course of this year are:

1) The results obtained with radio-calcium are, so far, identical to those obtained with radio-phosphorus. The precision of the autographs is somewhat better, however, and, therefore, radio-calcium seemed to be the isotope of choice for study of the development of the mineral elements of the tooth. On the other hand, this work has to be extended to mature teeth.

2) The sectioning of mature teeth has been considerably improved by the machine developed with Mr. Weiss' collaboration. Two photographs are enclosed which were obtained from material sectioned by the slicing machine. The first figure shows a cross section through the jaw of a young rat at 2 days after injection of radio-active calcium. This material was coated with photographic emulsion according to the method described in previous reports. The result is very clear. There is an overall autographic reaction of the bone which predominates in the periosteal region. On the other hand, the sections through the teeth appear as rings with a diffuse reaction. The inner edge of the ring is an intense

reaction which corresponds to the inner portion of the dentin. The results are not yet perfect when working with older animals. The second figure gives some idea of the numerous artefacts which are still encountered with the technique. The second figure represents a longitudinal section of an incisor tooth of a fully-mature rat. A definite reaction is seen in the inner portion of the dentin, as indicated by the arrow (D).

3) The results obtained with radio-carbon confirm that this radio-element is fixed in the organic substance in young animals to a far greater extent than in the inorganic substance. Further work is in progress on this subject.

September, 1952.

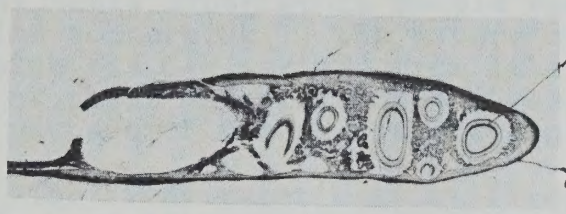

Fig. 1. Radioautograph of a section through the jaw of a young rat given radio-calcium.

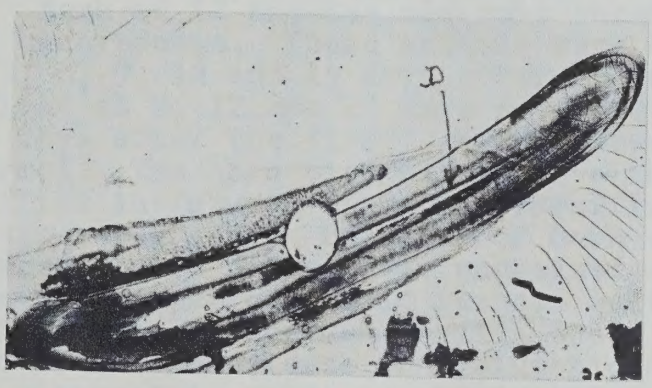

Fig. 2. Radioautograph of a longitudinal section of an incisor tooth of an adult rat.

APPENDIX "K"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1952

ENTRY OF RADIO-CARBON INTO TEETH

(Research in progress, R. C. Greulich)

Two separate experiments are in progress at this time. The results of these experiments should yield a considerable bulk of information concerning 1) the nature of the materials in dentin and enamel which fix C^{14} derived from labelled sodium bicarbonate; 2) the fate of these materials once formed; and 3) the effects of age upon the pattern of C^{14} fixation in enamel and dentin.

The first experiment utilizes the radio-autographic technique. Each of 12 one-day old rats received 30 μc of C^{14}-labelled sodium bicarbonate. These animals were then sacrificed in pairs at 1, 3, 6, 9, 12 and 15 days after injection. Those animals sacrificed at 9, 12 and 15 days received a second injection of 30 μc two days after the first. Hard tissues were fixed in alcohol-formol. The hard tissues of one animal of each pair were then decalcified in formate-citrate. Paraffin sections were prepared for radio-autography. Sections of both decalcified and undecalcified hard tissues were either left unstained or were stained with hematoxylin-eosin, periodic acid fuchsin sulfurous acid (PA-FSA) or safranin. Some of the hematoxylin-eosin stained sections were treated with ribonuclease or hyaluronidase prior to staining, to remove ribonucleic acids or chondroitin sulfuric acid, respectively. Some of the PA-FSA stained sections were treated with beta-amylase prior to staining to remove glycogen and starch. A total of 360 slides were used for histological study and radio-autography.

Since the beta-particle energy of C^{14} is very low, and since the absolute quantity of C^{14} present in a tissue section is minute, very long exposure times (up to a year) are necessary to yield satisfactory radio-autographs. However, it is hoped that when these radio-autographs become available, it will be possible to determine the fate of fixed C^{14} with time, and also to derive some information as to the nature of the materials containing C^{14} found in the dentine and enamel. More information, as well can be derived concerning the relative amounts of C^{14} incorporated into the organic and inorganic fractions of enamel and dentin.

As a corollary to this experiment and that reported below, an experiment is now in progress to determine by chemical means the changes in C^{14} uptake by the organic and inorganic fractions of teeth at different ages. Pairs of young, young adult and adult rats were injected with 30 μc $NaHC^{14}O_3$, and one of each pair was sacrificed twenty-four hours later. The remaining three animals were sacrificed seventy-two hours later. The incisors of these animals were removed and fixed in alcohol-formol.

ENTRY OF RADIO-CARBON INTO TEETH (cont'd)

R.C. GREULICH

Specific activity determinations of C^{14} in both the organic and inorganic fractions of enamel and dentin will be made. A comparison of these specific activity determinations at different ages should yield considerable information as to the effects of age on the pattern of C^{14} fixation in these structures. At present, work is in progress to establish a reliable technique for carrying out these determinations. It is hoped that it may also be possible to utilize paper chromatography combined with radio-autography to determine exactly the chemical nature of the materials in the organic matrix of enamel and dentin responsible for C^{14} fixation.

The first draft of a paper on the nature of the C^{14} fixed in teeth follows.

APPENDIX "K"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1952

ENTRY OF RADIO-CARBON INTO THE ORGANIC MATRIX OF GROWING TEETH

(Mr. R.C. Greulich)

Previous investigations have demonstrated conclusively that isotopes of the elements normally found in the mineral fraction of teeth can readily be traced in vivo to that fraction both by chemical and by radio-autographic techniques. Radio-autography has been employed to visualize the entry into teeth of those elements constituting the apaptite-like crystalline unit of the inorganic tooth fraction, namely phosphorus (Bélanger and Leblond, '50, and others), calcium (Minder and Gordonoff, '52) and carbon (Armstrong et al, '48).

Last year, in a general survey of the entry of C_{14} into tissues (Greulich and Leblond, '52a), it was briefly mentioned that C_{14}-labelled sodium bicarbonate injected into very young rats could also be incorporated into the organic matrices of enamel and dentin. The evidence for this suggestion was that hematoxylin stained preparations of teeth gave striking radio-autographs in dentin and enamel even though it was demonstrated that the acidity of the hematoxylin solution employed was sufficient to remove all of the inorganic material present.

These results, however, gave no indication as to the extent of incorporation of C_{14}-bicarbonate into the organic material relative to its incorporation into the inorganic fraction. Hence, a systematic study has been designed utilizing the radio-autographic technique and chemical techniques to determine qualitatively, by radio-autography, and quantitatively, by chemical analyses, the relative proportions of C^{14}-bicarbonate entering the organic and inorganic fractions of bone and teeth. The purpose of this report is to present the qualitative data obtained by radio-autographic studies. Work on the chemical analyses of C^{14} content of bone and teeth is now in progress.

Albino rats were injected at birth with C^{14}-labelled sodium bicarbonate and sacrificed 4, 24 and 72 hours later. Unstained sections of alcohol-formol fixed bones and teeth were prepared and half of the sections were decalcified in formate-citrate until control sections were no longer positive to the von Kossa reaction and no evidence of the presence of bone and tooth salts could be demonstrated by examination in polarized light. The undecalcified and decalcified sections were radio-autographed by the coating technique and, after exposure, were developed. All of the sections were coasted at the same time, given the same exposure time and development time.

Microscopic observation of the resultant bone and teeth radio-autographs revealed that little or no diminution in intensity of the reaction had occurred because of decalcification. This fact suggested that the bicarbonate ion is more or less preferentially utilized by the young organism in the synthesis of new organic matrix in the process of growth, and only secondarily utilized in the formation of the inorganic fraction of bone.

MATERIALS AND METHODS

Ten newborn albino rats of the Fisher strain were used in the experiment. The animals received the labelled bicarbonate approximately 12 hours after birth. Six of the animals were given a single subcutaneous injection of approximately 20 μc of C^{14}-bicarbonate, while the remaining 4 received approximately 40 μc of C^{14}-bicarbonate by the same route. The animals were sacrificed by means of chloroform at three time intervals after injection, namely, 4 hours (two animals given 20 μc and two given 40 μc), 24 hours (two animals given 20 μc and one given 40 μc) and 72 hours (two animals given 20 μc and one given 40 μc).

Bones and teeth were fixed in a 3:1 mixture of 95% alcohol and neutralized commercial formalin. These tissues were then dehydrated in dioxane, mounted in paraffin, and sectioned at 5 μ.

Several slides were prepared from each block. Half of these were decalcified by immersion in mixture of formic acid and sodium citrate (30 parts 85% formic acid to 44 parts 20% sodium citrate) until control sections no longer exhibited positive von Kossa reactions and no evidence of the presence of bone and tooth salts could be demonstrated by examination in polarized light. As a further control, undecalcified sections were stained by the von Kossa technique and also examined in polarized light to make certain that the bone and tooth salts had not been lost during fixation or embedding.

These unstained decalcified and undecalcified preparations were then radio-autographed by the coating technique (Gross et al,'51), utilizing NT4 emulsion. The duration of exposure was 4 months at -17°C. All of the procedures, including coating, exposure and development, were carried on simultaneously in order to eliminate any possibility of variations in technique influencing the intensities of the resultant radio-autographs. Following exposure and development, the preparations were subjected to routine fixation, dehydration and mounting in Canada balsam.

RESULTS

Figures 1 and 2 illustrate respectively the von Kossa stained preparation and the unstained radio-autograph of adjacent undecalcified sections of tibia and mandible. In the von Kossa preparation, sites of inorganic salt deposition are indicated by a black precipitate of metallic silver. These are seen in the mandible deposited on the alveolar bone, the developing dentin and enamel of the unerrupted incisor and molar, and as well, in the tibia, on the epiphyseal bone spicules, secondary epiphyseal ossification center and diaphysis. The corresponding unstained radio-autograph (fig. 2) exhibits striking reactions in the von Kossa positive regions of figure 1, as well as in non-dental and non-osseous tissues.

Figures 3 and 4 illustrate respectively the von Kossa stained preparation and the unstained radio-autograph of adjacent decalcified sections of tibia and mandible taken from the same block as those in figures 1 and 2. The absence of metallic silver deposits in any of the tissues stained by the von Kossa technique (fig. 3) indicates that the decalcification process employed had gone to completion. The corresponding

radio-autograph, however, still exhibits an intense reaction of the von Kossa positive areas shown in figure 1. The intensity of the radio-autographic reaction in figure 4 is remarkably similar to that of the corresponding undecalcified preparation (compare figs. 2 and 4). It should be noted that in preparing illustrations of the radio-autographs used herein, the exposure times used both for taking and printing the photographs were exactly identical. Similarly, the development time of the prints was kept constant. Therefore, little extraneous variation between the intensities of the two preparations was introduced by these processes.

These figures are only representative of the overall results, being taken from an animal sacrificed 24 hours after injection of approximately 40 μc $NaHC^{14}O_3$. However, it can be stated categorically that, in all of the animals, and at all of the time intervals, little or no diminution in the intensity of autographic reaction of bone and teeth as the result of decalcification could be demonstrated.

DISCUSSION

The radio-autographic technique lends itself well to the localization of specific tissue constituents. Under conditions of optimal exposure, other factors being equal, the intensity of the autographic image is proportional to the amount of radio-active material present. Hence, if any radio-active material is removed from a tissue section, the site from which it has been removed and a rough estimate of its amount can be assessed by comparison of treated and control sections.

In this experiment, sections of bone and teeth were completely decalcified, and, along with undecalcified sections, were autographed under essentially similar conditions. While no quantitative comparison of the intensities of the resultant radio-autographs was made, qualitative estimates indicated that the process of decalcification had little effect upon the intensity of the autographic reactions. It can be assumed, then, that very little of the radio-active carbon introduced into the organism found its way into the inorganic component of bone and teeth, whereas the greatest share of incorporation seemed to be in the organic fraction.

These findings are not in agreement with those of Armstrong et al (148), who demonstrated that in adult rats a far greater share of injected C^{14}-labelled carbonate ion finds its way into the inorganic fraction of bone and teeth. The discrepancy between these results probably represents the age differences between the experimental animals utilized for the two experiments. Armstrong and his group used old adult rats, judging by the body weights (400 to more than 600 gm), whereas our oldest animals were under 4 days of age at sacrifice (body weight < 10 gm). Certainly one would not expect the carbonate ion, once introduced into the bodily environment, to be treated in a manner different from bicarbonate ion (unless the dose administered was great enough to cause an alkalaemia of considerable duration).

In the very young animals used in this experiment, growth and formation of new tissue components is proceeding at a very rapid rate. All of the soft tissue of these animals exhibit radio-autographic

reactions, indicating the participation of the tagged atom from C^{14}-labelled bicarbonate in the synthesis of high molecular weight organic compounds (Greulich and Leblond, '52b). Since, at this time in development, the bones and teeth are among the most rapidly growing structures in the body, it is not surprising that the tagged carbon atoms are utilized in the formation of new organic matrix for these structures.

In adult animals, such as were used by Armstrong and his group, growth occurs at a markedly reduced rate. Thus, the de novo formation of new tissue constituents would not play an important role in the rate of incorporation of labelled carbon atoms. Apparently, the factors determining the incorporation of C^{14}-bicarbonate in the bones and teeth of such animals are 1) the rate of exchange of the inorganic components; and 2) the rate of turnover of pre-existing organic matrix. Armstrong did demonstrate a very slight incorporation of C^{14} into the protein fraction of teeth and bone, but this was far smaller than the amount incorporated into the inorganic fraction. It seems that in the adult, the rate of incorporation of C^{14}-bicarbonate or carbonate is greatest in exchange processes, and incorporation due to the formation of new organic matrix is much reduced. On the contrary, the formation of new organic matrix during growth accounts for the greatest share of C^{14}-bicarbonate incorporation in young animals, and processes of exchange of inorganic material are less important as a mechanism for C^{14} fixation.

SUMMARY

In order to determine the extent of incorporation of C^{14}-labelled bicarbonate into the organic matrix of teeth and bones, newborn albino rats were injected with 20 µc or 40 µc of $NaHC^{14}O_3$ and sacrificed at intervals of 4, 24 and 72 hours after injection. Unstained sections of alcohol-formol fixed bones and teeth were completely decalcified with formate-citrate. These decalcified sections, along with undecalcified control sections were radio-autographed by the coating technique under identical conditions.

Examination of the resultant radio-autographs revealed that little or no diminution in intensity of reaction could be attributed to the process of decalcification. This indicated that nearly all of the injected isotope localizing in the bones and teeth had found its way into the organic matrix rather than the inorganic constituents. This result was obtained in all animals at all time intervals after injection.

A comparison of these results with those of Armstrong et al ('48), suggests that marked differences exist between young and old rats in the hard tissue metabolism of bicarbonate and carbonate ions. Armstrong and his group showed in adult rats than injected carbonate ion labelled with C^{14} was fixed principally in the inorganic fraction of bone and teeth. Our results, in young animals, suggest that under conditions of rapid growth and concomitant formation of new tissue constituents, the C^{14} of labelled bicarbonate is fixed preferentially in the organic matrix of hard tissues and only secondarily in their inorganic fractions.

ACKNOWLEDGEMENT

This work was supported by a grant from the Associate Committee on Dental Research of the National Research Council.

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PLATE 1

EXPLANATION OF FIGURES

All figures are sections of the same tibia and mandible taken from a rat injected at birth with approximately 40 µc $NaHC^{14}O_3$ and sacrificed 24 hours later. Alcohol-formol fixation, 5 µ paraffin sections.

1 Undecalcified section stained by the von Kossa technique to demonstrate the presence of bone salts (phosphates). Blackening indicates a positive reaction.

Note the areas of blackening at the epiphyseal plate and in the shaft of the tibia, and, in the mandible, over alveolar bone and newly-formed dentin and enamel in the unerrupted incisor and molar.

2 Undecalcified and unstained section radio-autographed by the coating technique. In this case, the areas of blackening demonstrate the sites of C^{14} fixation. NT4 emulsion.

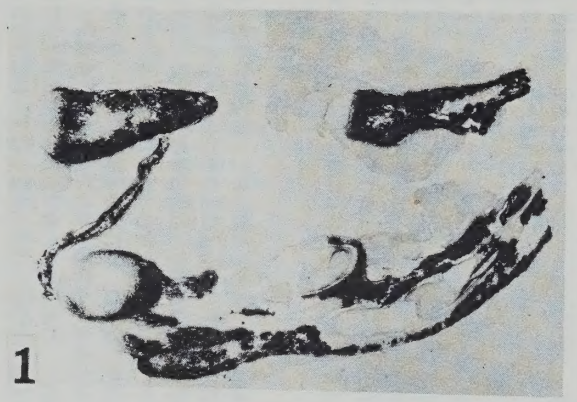
Note the general correspondence of the radio-autographic and von Kossa reactions in the hard tissues. The reaction of newly-formed dentin and enamel in the incisor is particularly marked.

3 Decalcified section stained by the von Kossa technique to demonstrate the presence of bone salts (phosphates).

No black precipitate indicative of the presence of bone salts is demonstrable.

4 Decalcified, unstained section radio-autographed by the coating technique. As in figure 2, the areas of blackening demonstrate the sites of C^{14} fixation. NT_4 emulsion.

Note the almost complete lack of reduction of radio-autographic intensity even though the section has been decalcified. As in figure 2, the newly-formed dentin and enamel exhibit a striking radio-autographic reaction.



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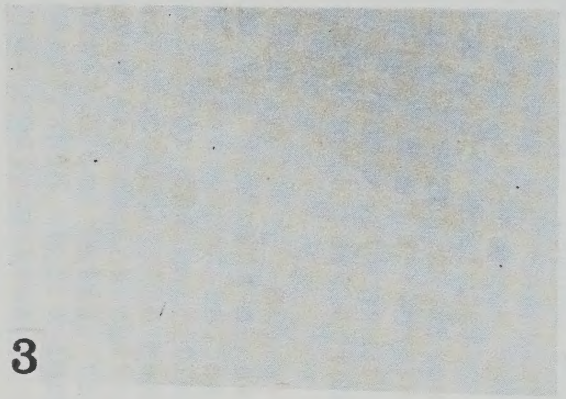

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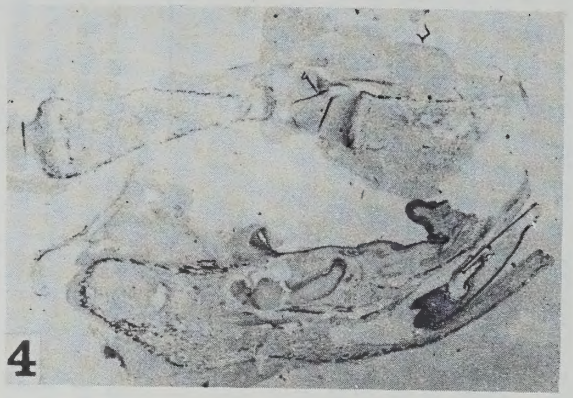

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APPENDIX "K"

DEVELOPMENT OF RAT TEETH STUDIED WITH CALCIUM 45

(Y. Kumamoto)

The deposition of radioactive phosphorus in the growing teeth of rats and hamsters was examined by the radioautographic technique (Bélanger and Leblond). Similar studies with radioactive calcium were carried out to find out whether the deposition of calcium was similar to that of phosphorus or not. In either case, important conclusions would be drawn regarding the growth pattern of the tooth.

It was particularly important to work with undecalcified teeth. This is easy in very young animals, but becomes extremely difficult as soon as the teeth become hard. In rats, the teeth of 10 day old animals are no longer easily sectioned. Arnold has described a method to cut very thin sections of mature bones using plasticized celloidin. It was, therefore, hoped that teeth could be sectioned by this method at least in older animals than now possible. An attempt is therefore being made to follow the development of teeth of rats beyond the tenth day of life using Arnold's technique.

MATERIAL AND METHODS

To follow calcium 45 administered subcutaneously as $Ca^{45}Cl_2$ to newborn rats, the following method was employed. Newborn rats were injected with $Ca^{45}Cl_2$, the dose being 1-0.6 microcuries per gram of body weight. The animals were nursed by the mother and were sacrificed at 3 hours, 1,3,6,9,12,15,18 days after injection. Lower jaws were removed for observing the incisors, upper jaws for molars. (Bones were also removed). Teeth of animals of six days and under were sectioned by the paraffin method. However, teeth of older animals required the use of plasticized celloidin, consisting of 1/2 sec nitro cellulose, 11 parts, diamyl phthalate, 9 parts, and acetone (Arnold). The addition of the plasticizer, diamyl phthalate, made the celloidin block less brittle and hence allowed sectioning of older teeth. The sections of all animals were stained with alizarin and radioautographs were made by the coating method (Gross et al.).

Two points required attention in the staining of sections of calcified tissues, especially for those which contained radioactive calcium. First, the stain should be specific for the calcified tissue, and second, such stain should not remove anv calcium. The commonly used hematoxylin and eosin stains not only removed the radioactivity due to the acidity of the hematoxylin solution, but stained mainly the soft tissues. Other stins were tried, namely, saffranin, basic fuchsin, and alizarin. Alizarin (0.1% in 95% alcohol) was most successful as indicated below.

Sections of teeth and bones of rats injected with calcium 45 were mounted onto slides and the radioactivity measured by holding the tube of the Geiger counter over the sections. These were then stained with basic fuchsin and alizarin and the radioactivity measured again. The dehydration after the staining was followed by a coat of 1% celloidin which is used in protecting the sections to be coated with emulsion later. Counts were taken after the staining and were then radioautographed.

Sections of celloidin and plasticized celloidin embedded material were also tried. Sections obtained from the same rats as the above were stained with saffranin which stained cartilage but not teeth or bones. These were not counted before staining but radioautographs were made.

Observations and Results

All incisors and molars were cut longitudinally.

Two and three hours after injection some calcium 45 is distributed diffusely throughout the dentin and enamel of the incisor, but the former has more Ca 45 than the latter. Autographic reaction seemed heavier nearer the tip of the incisor, than proximally. Molars were found developing at different stages; however, results of the molars, if obtained at all, were similar to those of the incisors.

Twenty-four hours after injection of Ca^{45}, the enamel of the incisors showed a faint general reaction. The dentin showed reaction throughout the whole incisor, being heaviest midway between the odontoblasts and the dentino-enamel junction at the apical end, whereas basally (i.e., the root of the tooth), the reaction was throughout the whole thickness. On the lingual side of the incisor, the dentin showed a general reaction throughout the whole thickness and length. In the molars, reaction was diffuse throughout, with more in the dentin than in the enamel.

Three days after the injection, reaction in the enamel of the incisor was distributed throughout but increased apically. Along the basal half of the dentin, on the labial side, the reaction was heaviest at the dentino-enamel junction, but apically, the site of heaviest reaction is slightly away from the junction. Molars still showed various degrees of development, some had not even begun to calcify. Those which were calcifying, showed a general reaction throughout the thickness, heavier apically than basally.

Six days after the injection, the enamel of the incisors showed denser reactions than in the younger rats but still the reaction was general throughout the whole width of the enamel but lighter than dentin. However, the autographic reaction was found to be the heaviest in the apical third and practically none in the basal third. The dentin showed a heavy reaction being at the dentino-enamel junction up to about midway along the length of the dentin, while apically the site of heaviest reaction was slightly away from the junction. The dentin on the lingual side, which is exposed in the rat, the line of heaviest reaction marks the periphery of the dentin. The molars showed much the same pattern as in the 3 day old rats.

These radioautographs showed that after the initial diffuse reaction had disappeared, the intensity of the autographic reactions remained about the same, showing little decrease with time. From this observation it may be safely concluded that Ca^{45} is incorporated in a permanent form in thecalcified structures. Also the site of the reactions does not change with time although it is closer to the dentino-enamel

junction relatively speaking, since the structures grew since the time of injection and the time of sacrifice. Similar results have been reported with radioactive phosphorus experiments (Bélanger & Leblond).

Radioactivity counts taken after the staining showed only a very slight decrease for the sections stained with alizarin and an even slighter decrease for those stained with basic fuchsin. Examination under the microscope showed that while alizarin coloured the calcified areas a deeper shade than soft tissues, basic fuchsin stained the soft tissues, leaving the calcified areas relatively unstained. Autographs of these sections showed much the same intensity of reaction on the photographic silver. These sections were those of young rats up to six days and were sectioned in celloidin and these stained with alizarin also showed no decrease in radioactivity. In saffranin stained sections, the radioactivity was leached out during the staining and the autographic reactions when obtained were very light.

It was concluded that alizarin staining fulfilled the requirements of staining calcified tissues and at the same time leaving the radioactivity relatively intact. Therefore in subsequent work involving sections of calcified tissues containing Ca^{45}, this dye was adopted as a routine stain.

Plasticized celloidin technique possessed the advantage of a more cohesive, elastic embedding material. However, in the preliminary experiments with this new medium, a number of difficulties arose, which to date have not been completely solved. There seems to be a limit to the durability of the cutting edge of the microtome knife when hard calcified tissues, such as teeth are being sectioned. This limit is tentatively observed to be when teeth of rats are 12 days old. Secondly, the block of plasticized celloidin with the tissue is much harder than ordinary celloidin blocks and when the carriage of the microtome holding the knife is advanced over the sections, there is often the tendency to ride over the block. This is probably due to the play found between the microtome knife carrier and the slideway. (The sliding microtome made by American Optical Company was used.) This of necessity, limits the thinness of the sections obtainable. At present, six and four micron sections have been tried on molars of 9 and 12 day old rats but there is much to be improved. Work is continuing on the subject.

CONCLUSIONS

(1) In conclusion, the deposition of radio-calcium in the teeth rats up to 9 days of age was examined and found to be similar to that after radio-phosphorus treatment.

(2) Methods are being devised to extend the experiment to older animals.

"K-4"

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APPENDIX "K"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1952

SECTIONING OF ADULT TEETH

(Mr.M.Weiss)

The hardness of the tooth makes it more difficult to section than other organs. Thus, until now, no technique having anywhere near the simplicity, speed and precision of the microtome has been devised for making histological slides of undecalcified teeth. Research workers have not been unaware of the problem. From as far back as 1678, when Anthoney van Leewwenhoek first studied the microscopic structure of teeth, until today, a vast number of techniques have been devised to cope with this problem. Tooth sections were usually made by grinding and polishing, a procedure which is not quite satisfactory. An attempt was made to build a mechanical device incorporating recent industrial techniques and having sufficient flexibility to allow for the alteration of the technique as experience makes it desirable.

Recently, Atkinson (1950) was able to produce sections 25 micra thick by slicing with a 25 micra-thick carborundum wheel (without any polishing required unless it was desired to make sections thinner than 25 micra). To build a complete machine like that of Atkinson's with the degree of precision required would have been the equivalent of building a microtome for the first time. It was decided to adapt the Black and Decker Valve resurfacing machine. After certain alterations, a few 25 micra-thick sections were produced, as indicated in last year's report.

It was found desirable to embed the teeth in methacrylate, since by this technique the soft and hard tissues are preserved in their normal relationship. Also, this technique helped simplify mounting of sections. The commercial methyl methacrylate is prepared by shaking up three times with N sodium hydroxide to remove the inhibitor; the liquid, after separation from the sodium hydroxide, is dried over anhydrous sodium sulfate and filtered. Benzoyl peroxide (catalyst) up to 5% is added, and the liquid again filtered. The liquid will polymerize any time up to 8 hours at 4°C.

Another recent improvement to simplify the work with the machine was to make the feed automatic. Thus presently the operator only has to make the adjustments after each slice is completed and turn on the switches for the cutting of the next section. A number of sections of human and rat teeth were produced by this method.

The actual cutting of the specimen is done by means of a thin carborundum wheel (A) mounted on a rapidly revolving spindle which is spring loaded and runs on sealed ball-bearings.

SECTIONING OF ADULT TEETH (Mr. M.Weiss) (cont'd)

The spindle and flanges (B) for holding the wheel are precision machined and run true within .0003 inches. The direction of rotation is towards the operator and is obtained by means of a V belt connecting the motor (C) and the spindle (D). The movement of the specimen (E), which is held in a clamp (G), is obtained by a coarse screw feed (H) and a fine micrometer feed (I). The first being used for the rough placing of the specimen and the latter for controlling the exact thickness of the section. Thus for obtaining sections 25 micra thick, the specimen is advanced by the micrometer feed the thickness of the wheel, 125 micra plus the thickness of the section, 25 micra; i.e., a total of 150 micra before the commencement of each slice.

A slide screw (J) controls the feed of the carborundum wheel through the block (E). This can be operated manually to position the block properly for the commencement of the cut; after this, the switch for the motor and gear mechanism (L), connected by a V belt to the other end (M) of the screw feed (J), is turned on. The cut is completed automatically. Water is used as the cutting lubricant and directed to each side of the wheel from spouts (R). The wheel is enclosed in a plastic guard (S) to protect the operator from radio-activity.

A major difficulty was to catch the sections as they were sliced off the block. A technique was devised of sticking a plastic cover slip with acetone on the uncut block before each slice is taken. Thus, when the section is sliced off it is already attached to a plastic cover slip. This plastic cover slip is attached onto a glass slide with the section exposed using a fast-drying plastic spray. This technique of using a plastic cover slip solves several problems relative to radio-autographic technique, since the section must have one surface free from any foreign material and still be mounted on a slide on the other side. One of the important functions of the cover slip during the cutting of the section is that it acts as a guide for the wheel, thus making possible the cutting of thinner sections. Also with any other technique for mounting, the sections would be distorted, an undesirable feature for radio-autographs.

The present machine offers a relatively simple technique for making ground sections, thus offering a valuable tool for the study of metabolism of the hard tissues of the body without the expenditure of too much technical work.

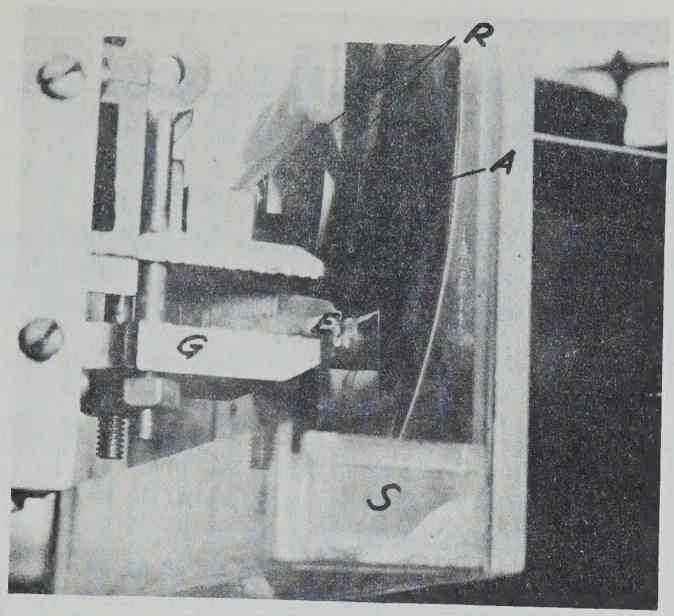


Fig. 1
Cutting wheel (A) and block to be sectionned (E). The plastic holder is attached

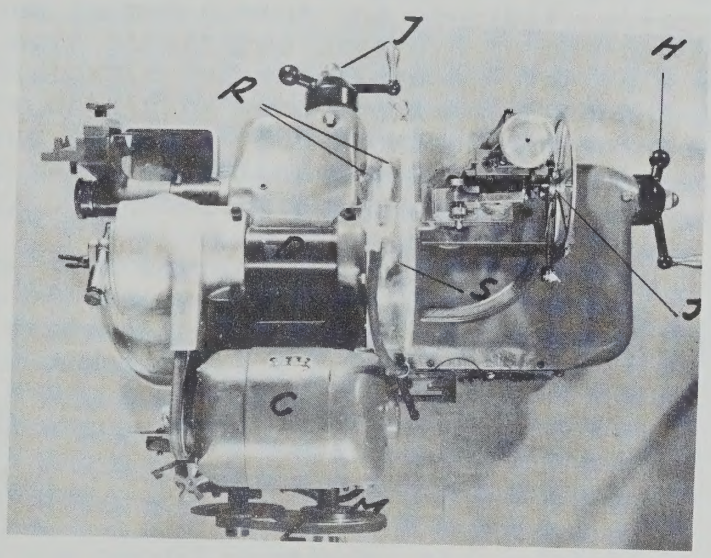


Fig. 2.
View from above of the whole machine

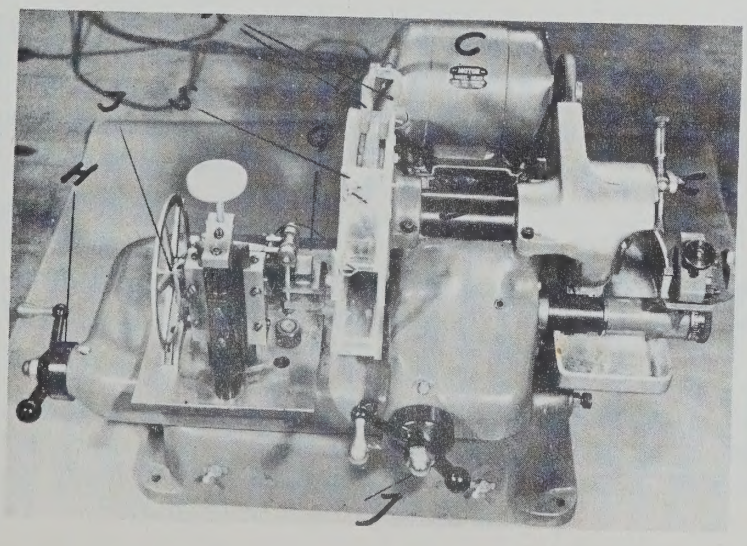


Fig. 3
Front view

APPENDIX "L"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1952

Subject: Sectioning of teeth and surrounding structures and the effects of potassium deficiency and of pyridoxine deficiency on the teeth and surrounding structures of dogs.

Grantee: Dr. A.G. Racey

Project No: DR 39 Amount of Grant $1600.00

SUMMARY OF WORK

In a previous report to the committee, the technical procedure was outlined and the problems encountered were enumerated. It was found that the gases formed during the decalcification process, tended to disturb the soft structures attached to the teeth and the alveolar bone and thus a distortion of the tissues. For this reason it is not an ideal apparatus for obtaining good sections of teeth, alveolar bone and soft tissues in one section. But it was found to be very effective when single teeth were used with no soft structures attached in order to study the pathology of dental pulp.

The work carried out so far this year has been confined mostly to the study of the pyridoxine deficient dog and the report is based on th microscopic study of sections cut in a longitudinal plane of similar teeth of normal and of pyridoxine deficient dogs.

The following observations were made.

1. EPITHELIUM:-

In the normal specimen, the epithelium is covered with a thick layer of keratin. The epithelial cells are well stained. (See Fig. 1.)

In the pyridoxine deficient animal, the keratin layer is thin and what is keratinized seems to be desquamated. The epithelium is not as thick as the normal and the epithelial cells do not stain as well. It does seem possible that the epithelium could be in the early stages of degeneration. (See Fig. 2.)

2. PERIODONTAL MEMBRANE:-

In the normal animal the periodontal membrane consists of dense collagen fibres with numerous fibrocytes and numerous blood vessels (See Fig. 3.)

In the pyridoxine deficient specimen, the fibres appear to be more definite than the normal, but do not appear to be as numerous. There are a greater number of blood vessels present and some evidence of edema. (See Fig. 4.)

3. ALVEOLAR BONE:-

In the normal specimen the alveolar bone is very dense and darker staining. (See Fig. 3.)

In the pyridoxine deficiency, there is evidence of osteoporosis of the alveolar bone. (See Fig. 4.)

4. DENTAL PULP:-

In the normal specimen, there are present blood vessels and fibrocytes and odontoblasts, etc. (See Figs.7 & 8.)

In the pyridoxine deficient animal, however there is an engorgement of the blood vessels with red blood cells. (See Figs.9 & 10) There is a lack of fibrocytes. In the odontoblastic layer there appears to be an incipient regressive change here. There is a sharp demarcation between the dentine and the odontoblastic layer and this is not so in the normal specimen. In the pyridoxine, predentine appears to be absent and the odontoblasts themselves appear to be in a process of degeneration. (See Figs.11 & 12.)

While it is realized that no definite conclusions can be made from such a limited amount of material, it is thought that the above are the main changes which have taken place in these structures of the pyridoxine deficient animal. These observations have been made on dogs obtained from the Department of Experimental Surgery. It is thought that much additional information would be gained from a study of young animals on the deficient diet from shortly after birth. This is to be done as soon as the necessary animals can be obtained.

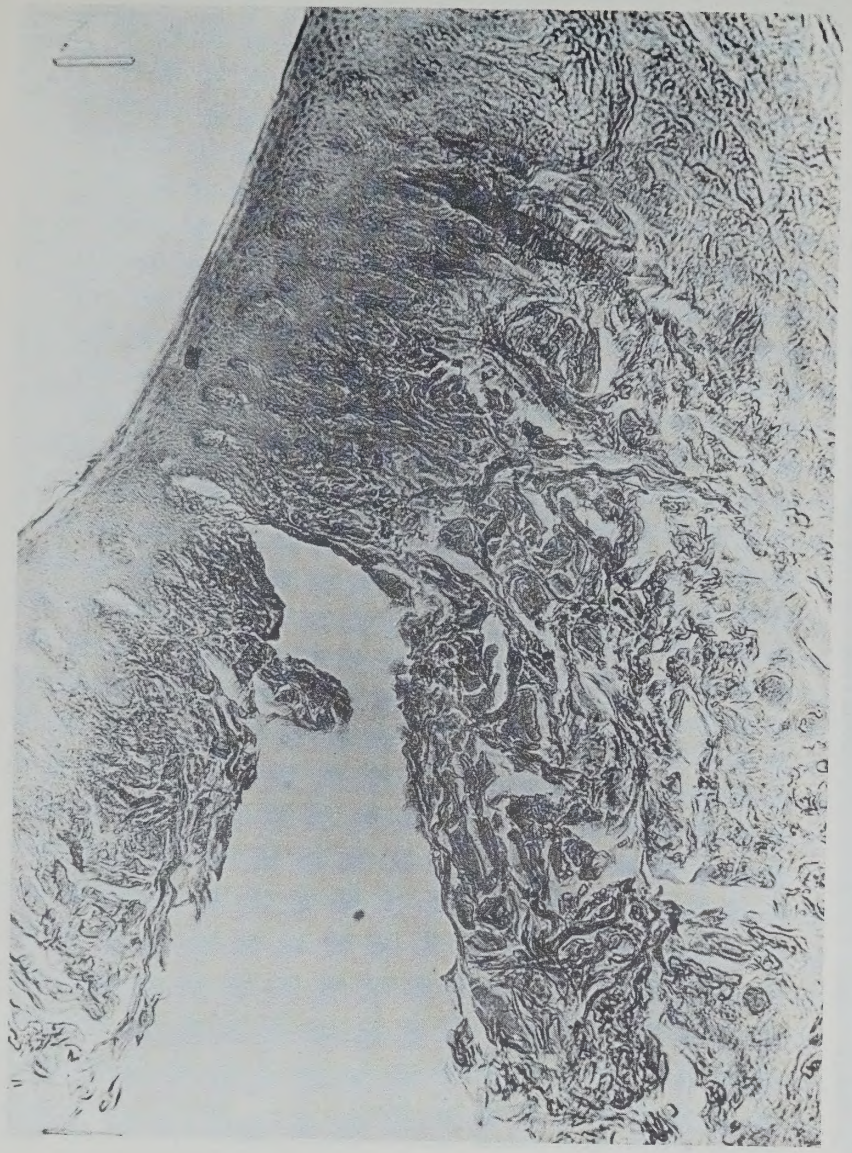


Fig. 1 Normal

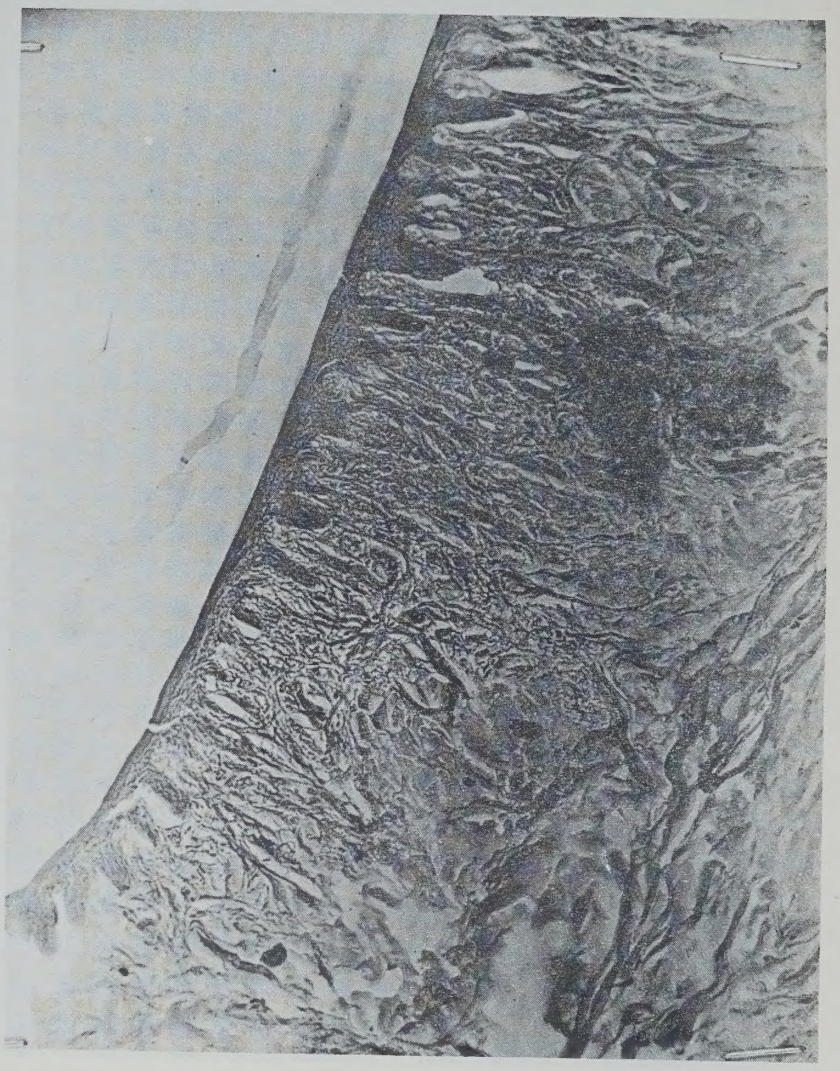


Fig. 2 Pyridoxine deficiency

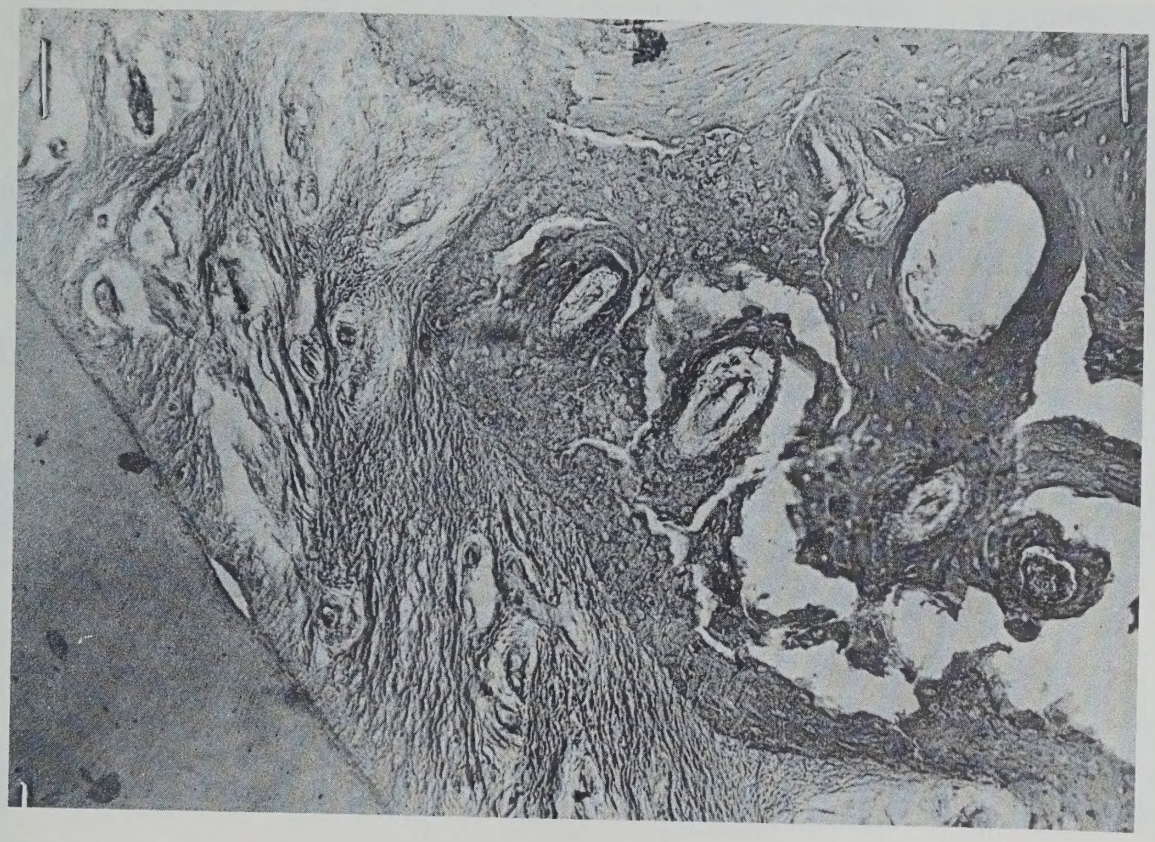


Fig. 4

Fig. 3

Fig. 7

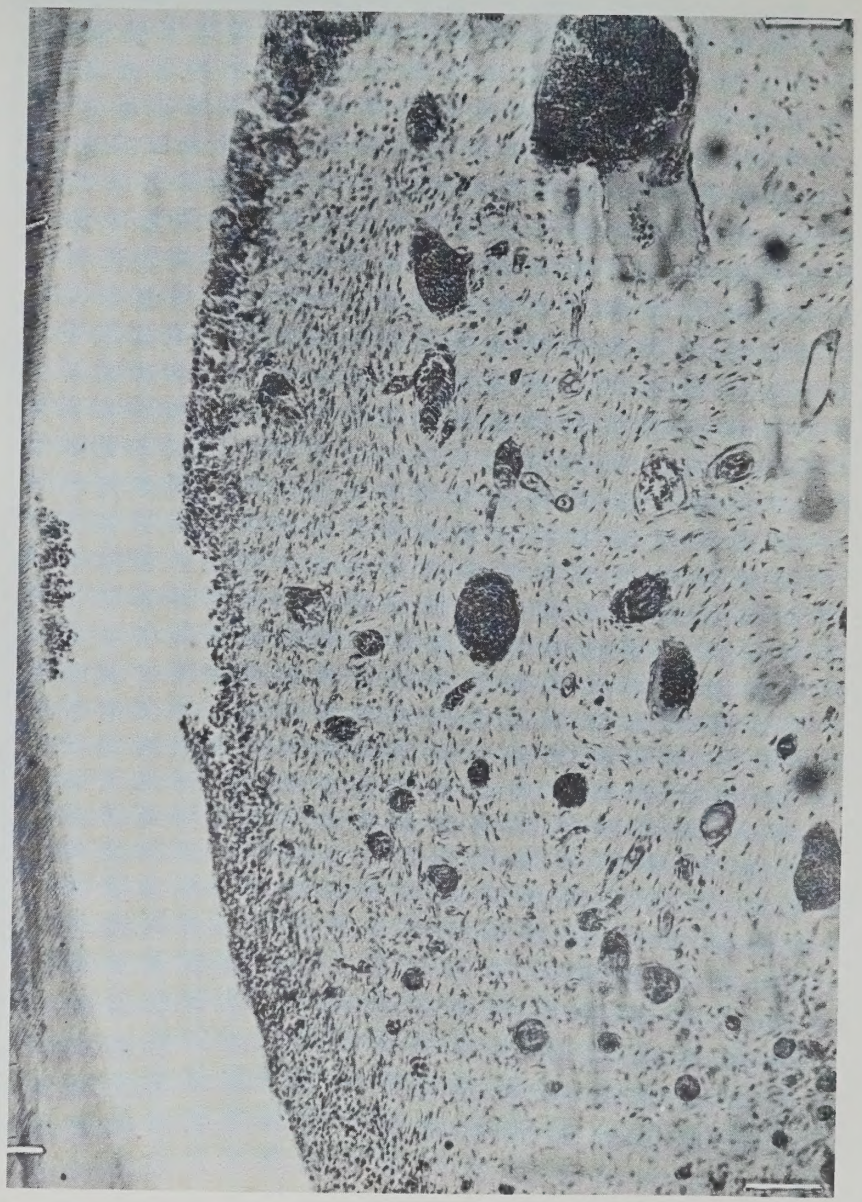


Fig. 8

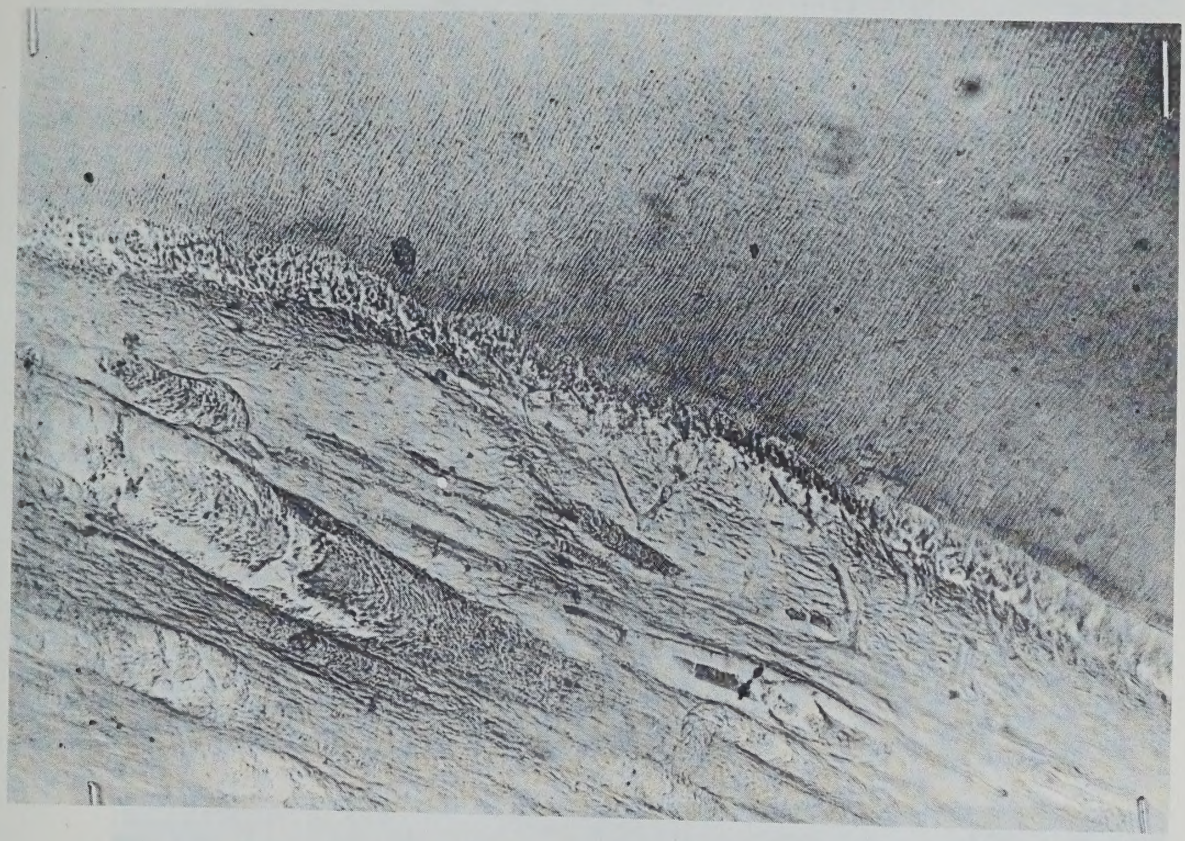


Fig. 10

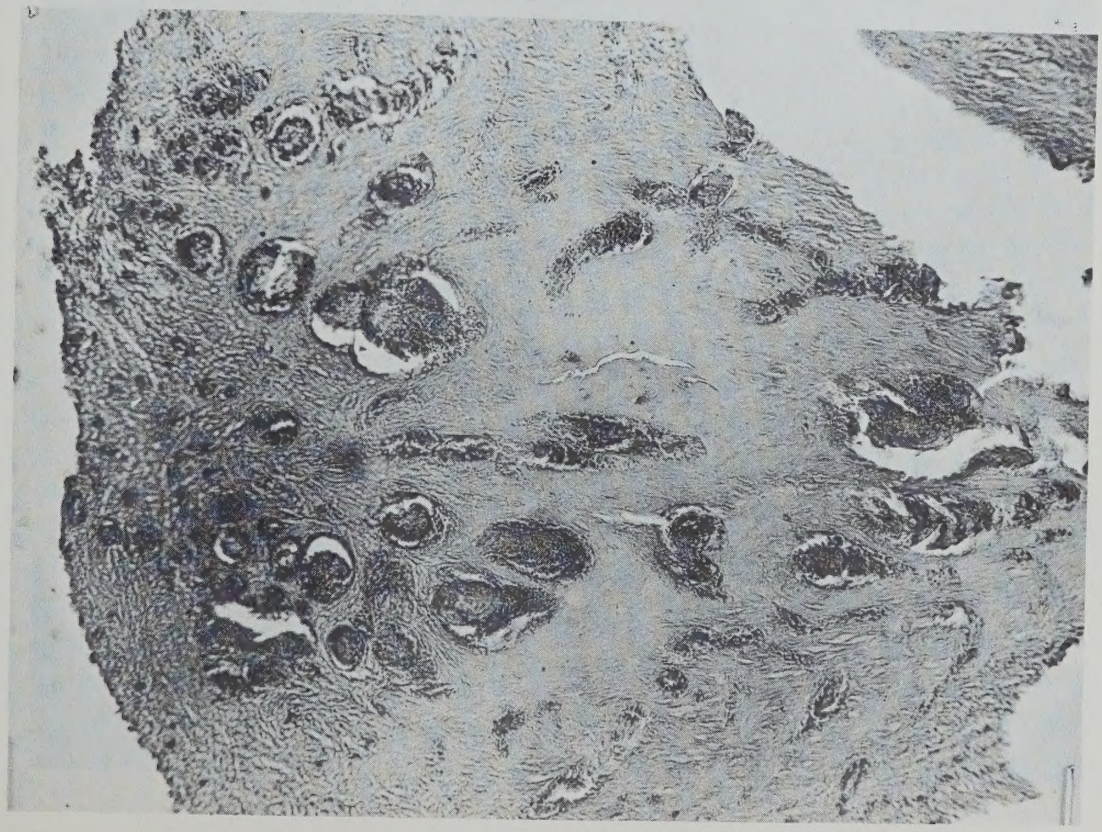


Fig. 9

Fig. 11

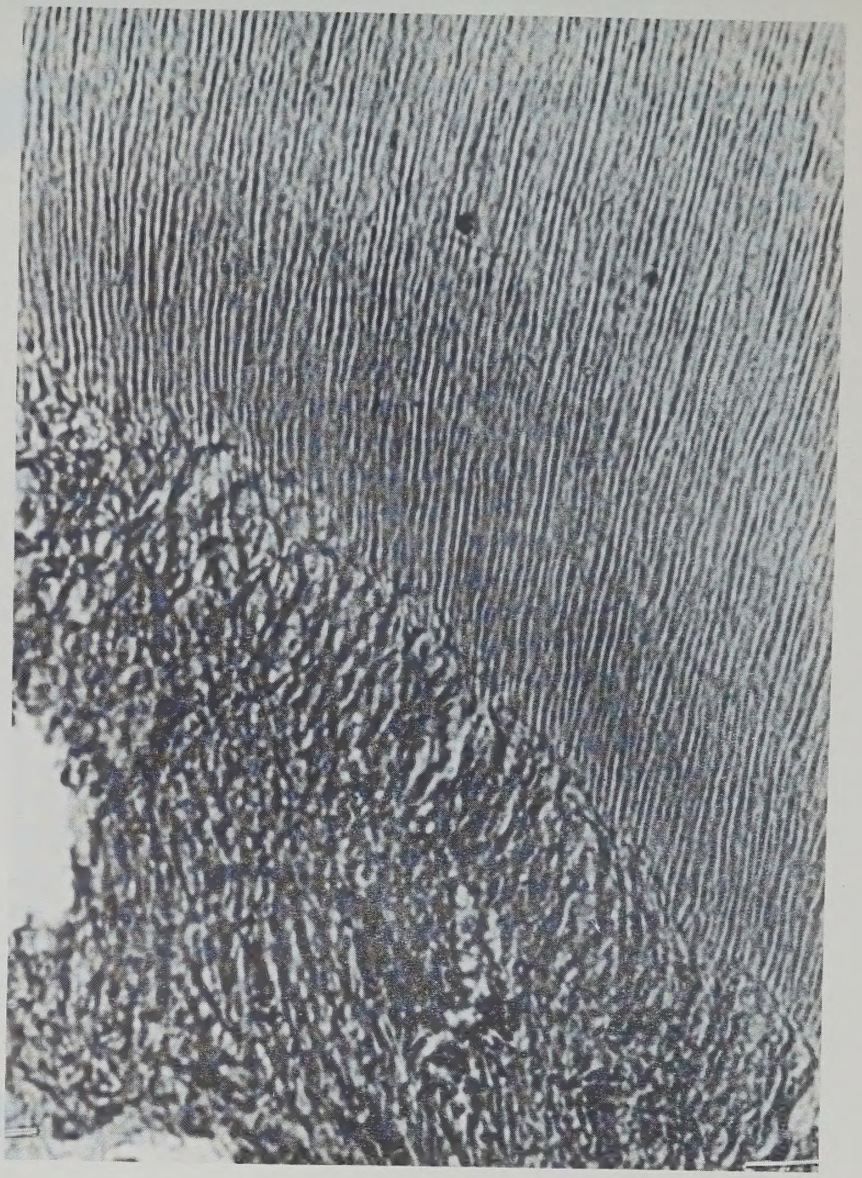


Fig. 12

Fig. 13 Normal Dental Pulp

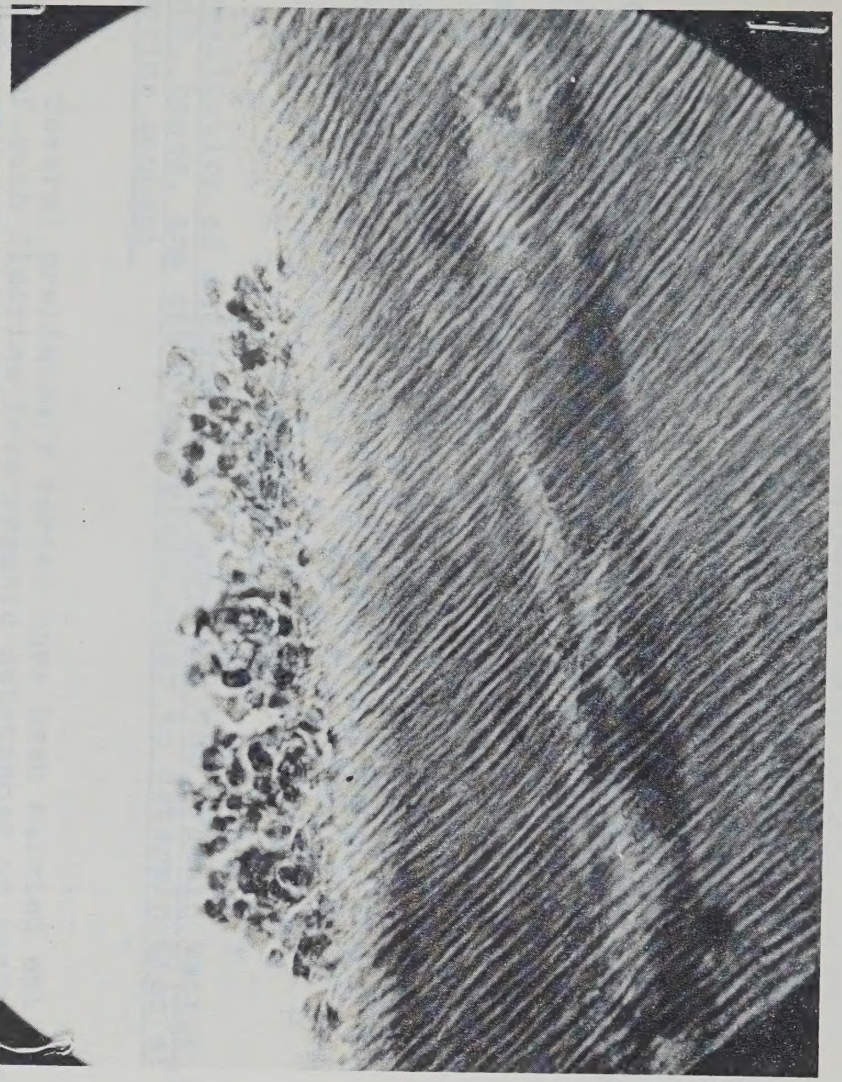
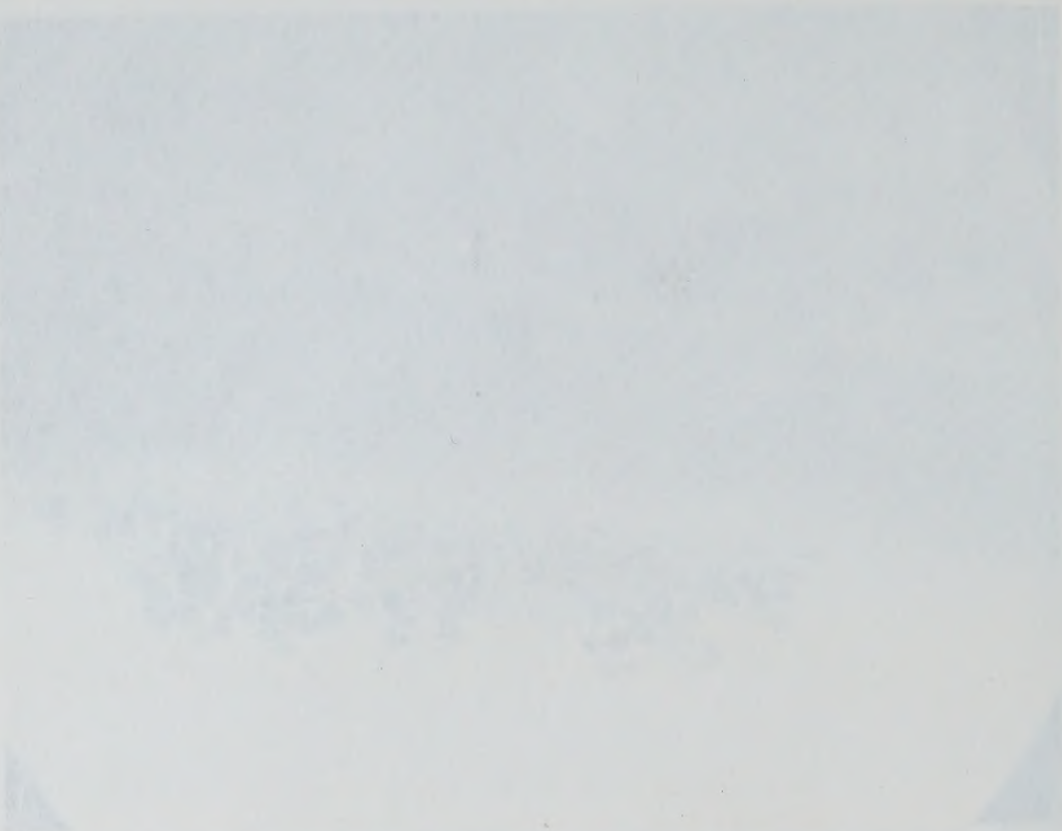


Fig. 14 Normal Dental Pulp



APPENDIX "M"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1952

Subject: Development of a method for determining fluorine by means of a photo-electric colorimeter.

Grantee: Dr. O.J. Walker

Project No.: DR 28 Amount of Grant $600.

SUMMARY OF WORK

Investigation of methods for destroying the organic matter in teeth, bones, and other materials prior to determination of fluorine content

Several preliminary series have been carried out on the ashing of such fluorine free organic substances as sugar and starch using such alkaline reagents as magnesium nitrate, calcium hydroxide, barium peroxide, and barium hydroxide. The ashing step was satisfactorily completed in about one hour. Additional experiments were carried out in the ashing of the same materials in the presence of the same reagents, and known amounts of fluoride, the products being dissolved in dilute sulfuric acid, distilled, and the fluorine in the distillate determined.

The recovery of fluorine varied in an irregular manner.

Conclusion. More experimental work is necessary in order that a satisfactory procedure be developed.

Date: September 16, 1952.

APPENDIX "M"

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September, 1952

Subject: Investigation of methods for destroying the organic matter in teeth, bones, and other materials prior to determination of fluorine content.

Grantee: Dr. O.J. Walker

Project No.: DR 28 Amount of Grant: $600

The destruction of organic matter in bones and teeth is essential prior to the determination of fluorides by a photoelectric colorimetric method. There is a loss of fluorides when an acid ashing is used, therefore, basic methods were investigated.

A series of ashings were carried out adding a known amount of fluoride as sodium fluoride to fluoride free materials, namely sugar and starch. However, the bulk of the work was done with 1 gm. samples of starch because it was found to be easier to handle, and less likely to boil over during the ashing. Nickel crucibles were used in all cases. The ashing, which was in two steps, required 15 - 30 minutes over low flame and wire gauze in most cases, but from 30 -60 minutes for magnesium nitrate, for preliminary charring and 30 - 45 minutes heating over a clay triangle for completion, using full heat of a Meker burner. Ashing at a lower temperature using Bunsen burners instead of Meker burners increased the ashing time but did not improve the fluoride recovery.

To the products of the ashing were added 110 ml. of distilled water and 40 ml. of concentrated sulphuric acid. The mixture was distilled using a 51 cm. condenser with ground glass joints, and the first 90 ml. were collected. No steam escaped and a cool distillate was obtained. Distillation took 15 - 20 minutes.

Several runs were made on teeth using magnesium nitrate. The tooth was dried in an oven for 3 - 4 hours at 110°, and powdered with an agate mortar and pestle. The values varied from 0.016% to 0.040% fluoride. The ashing method however, has not proven to be completely satisfactory, so that these results have little meaning.

The fluoride concentration in the distillate was determined by the sodium alizarin sulphonate photoelectric colorimetric method (1), which is based on the bleaching of the lake formed from a zirconyl salt and sodium alizarin sulphonate by the action of fluoride ion. A check was run on sulphate carried over in distillation since a concentration of 150 ppm or greater interferes with the results (2). Corrections were applied based on sulphate concentrations found using THQ (Tetrahydroxy-quinone) indicator.

a. Magnesium nitrate (3,4)

One ml of 20% magnesium nitrate solution was added as ashing agent. Although several runs were carried out, the following are representative of the general results.

mg. F^- added	mg. recovered	% recovered
0.00	0.008	100
0.10	0.104	104
0.15	0.100	67
0.30	0.356	120
0.40	0.400	100

Although some of the results appear to be fairly good there is no explanation for the discrepancies and there is no apparent sequence.

b. Calcium hydroxide

One ml of 5% calcium hydroxide suspension was added to 1 gm. of starch and known fluoride as ashing agent. The fluoride recovered decreases as the known quantity of fluoride added is increased.

mg. F^- added	mg. recovered	% recovery
0	0.112	100
0.20	0.292	145
0.40	0.400	100
0.45	0.440	98

Blank runs using calcium hydroxide indicate fluoride impurity in the reagent.

c. Barium peroxide

Barium peroxide added in approximately 10% the weight of the organic matter did not retain the fluoride as well as the reagents used previously. Results for barium peroxide + calcium hydroxide were similar to results for calcium hydroxide by itself.

d. Barium hydroxide

Two ml. of 5% barium hydroxide suspension (roughly equal to 1 ml. 5% calcium hydroxide was used in the same ashing procedure.

The following results show the general trend of the series of runs made.

	mg. recovered			% recovery
mg. F^- added	(1)	(2)	Ave	
0.05	0.12	0.08	0.10	200
0.15	0.13	0.15	0.15	100
0.25	0.26	0.27	0.26	104
0.35	0.24	0.20	0.22	63
0.50	0.29	0.35	0.32	64

The results were high for concentrations from 0.0 to 0.05 mg., and within experimental error from 0.10 to 0.25 mg., but at 0.3 mg. there seems to be a definite break and recovery of the fluoride is poor. Results using larger amounts of barium hydroxide, i.e. 1 gm. barium hydroxide to 1 gm. starch, and results using barium hydroxide + potassium permanganate were similar to those using the barium hydroxide suspension. However, a complete series was not run for these two methods.

e. Potassium hydroxide

Using potassium hydroxide as ashing agent, it appears, from a series of runs that this reagent is the least effective in retaining the fluoride.

Conclusion

The methods of alkaline ashing for destruction of organic matter investigated so far have proven to be unsatisfactory. There seems to be a general tendency to get greater than 100% values for low fluoride concentrations, but poor recovery for higher fluoride concentrations. More experimental work is necessary in order that a satisfactory procedure be developed.

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APPENDIX "N"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1952

Subject: A study of migration of tooth germs before and during mixed dentition.

Grantee: Dr. Willa Liu Chou

Project No.: Fellowship Grant Amount of Fellowship: $880

SUMMARY OF WORK

The practical investigation of this project has not been started because there is no series of X-ray records available in the Faculty of Dentistry, University of Toronto, and the investigator was not able to obtain the American visa to go down to University of Iowa for selecting her materials. In order that the investigator can finish the study in the near future, she is still trying hard to secure her visa.

In the past year, the investigator made a general review of literature, learned the cepholometric and tracing technique and studied statistical analysis of data to be used in the study.

A brief outline of the selection of materials and method of study are presented as follows:-

Materials: Lateral and posterior-anterior head X-rays of children from 3 to 13 years of age will be chosen. Overlapping in ages will be not avoided. At present the investigator intends to take one hundred cases for this study temporarily. The cases will be treated separately according to sexes and occlusal conditions. Since racial difference may be one of the causes of occlusal difference, the cases must be limited in one racial origin. Habits, physical conditions, genetical factors, etc., will be taken into consideration also.

Method: Series of tracings of the lateral and postero-anterior head X-rays of an individual taken in definite intervals will be used. Series of measurements will be taken from reference planes to three points on a tooth germ, i.e., the highest point of the crown, the middle point on the junction of the crown and the neck, and the end point of the formed root. The difference between each series of measurements will show the rate and direction of the migration of the tooth germ.

APPENDIX "M"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1952

Subject: A study of migration of tooth germs before and during mixed dentition.

Grantee: Dr. Willa Lin Chou

Project No.: Fellowship Grant Amount of Fellowship: $880

SUMMARY OF WORK

The practical investigation of this project has not been started because there is no series of X-ray records available in the Faculty of Dentistry, University of Toronto, and the investigator was not able to obtain the American visa to go down to University of Iowa for selecting her materials. In order that the investigator can finish the study in the near future, she is still trying hard to secure her visa.

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A brief outline of the selection of materials and method of study are presented as follows:-

Materials: Lateral and postero-anterior head X-rays of children from 3 to 13 years of age will be chosen. Overlapping in ages will be not avoided. At present the investigator intends to take one hundred cases for this study temporarily. The cases will be treated separately according to sexes and occlusal conditions. Since racial difference may be one of the causes of occlusal difference, the cases must be limited in one racial origin. Habits, physical conditions, genetical factors, etc., will be taken into consideration also.

Method: Series of tracings of the lateral and postero-anterior head X-rays of an individual taken in definite intervals will be used. Series of measurements will be taken from reference planes to three points on a tooth germ, i.e., the highest point of the crown, the middle point on the junction of the crown and the neck, and the end point of the formed root. The difference between each series of measurements will show the rate and direction of the migration of the tooth germ.

"N-2"

The migration of tooth germ in the alveolar bone may have five directions. The anteriors may migrate labially or lingually, mesially, or distally, and occlusally. At least, therefore, three reference planes should be chosen on the facial skeleton for showing the migration of tooth germ on the three dimensions in each stage.

The whole face grows forward, downward and laterally. Every point on the facial skeleton will move in any two of these three directions or in all of the three directions during the period of time from 3 to 12 years of age. It is impossible to establish a stable reference plane to study the migration condition of a tooth germ. Observing the difference in relative relation between a tooth germ and its surrounding bones seems to be the only method to approach. Since the upper and lower jaws may be different in growth and development, may be influenced by different genetical factors, and may be affected by different acquired factors, such as trauma and diseases,it is necessary to use different reference planes.

For observing the mandibular tooth germs, three reference planes will be chosen. They are 1, the tangent plane to the lower border of the mandible, 2, the perpendicular plane to the tangent plane of the mandible which must cut the most prominant point of the chin, and 3, a plane from the middle point between the orbits to the inter-dental alveolar crest of the deciduous or permanent central incisors. Three series measurements will be taken. Each series contains three perpendicular distance from the three points on the tooth germ to one of the above described reference planes.

Since the upper face is more complicated than the lower and the details of the bones in the tracing is not so clear as the lower, so the reference planes must be easily located, and have the least change in position. A line drawn through the upper margin of the hard palate is used for studying the migration of tooth germ in occlusal direction. The median plane is used for observing the mesial-distal migration of the anteriors and the buccal-lingual migration of the posteriors. A line drawn from the nasion to the deepest point on the anterior border of the premaxilla is used for the migration of the anteriors on labial-lingual direction and of the posteriors on the mesial-distal direction. Three series of measurements also will be obtained from these reference planes to each tooth germ.

25 September, 1952.

The whole face grows forward, downward and laterally. Every point on the facial skeleton will move in any two of these three directions or in all of the three directions during the period of time from 3 to 12 years of age. It is impossible to establish a stable reference plane to study the migration condition of a tooth germ. Observing the difference in relative relation between a tooth germ and its surrounding bones seems to be the only method to approach. Since the upper and lower jaws may be different in growth and development, may be influenced by different genetical factors, and may be affected by different acquired factors, such as trauma and diseases, it is necessary to use different reference planes.

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Since the upper face is more complicated than the lower and the details of the bones in the tracing is not so clear as the lower, so the reference planes must be easily located, and have the least change in position. A line drawn through the upper margin of the hard palate is used for studying the migration of tooth germ in sagittal direction. The median plane is used for observing the mesial-distal migration of the posterios and the buccal-lingual migration of the posterios. A line drawn from the nasion to the deepest point on the anterior border of the premaxilla is used for the migration of the anterios on labial-lingual direction and of the posterios on the mesial-distal direction. Three series of measurements also will be obtained from these reference planes to each tooth germ.

25 September, 1952.

APPENDIX "O"

A COMPARISON OF THE PATTERNS OF CLINICAL ERUPTION OF THE DECIDUOUS TEETH IN MAN WITH GROWTH IN WIDTH OF THE DENTAL ARCHES

BY

MARGARET ELIZABETH HATTON

A Thesis submitted in conformity
with the requirements for the degree of
Doctor of Philosophy
at the
University of Toronto

1952

SUMMARY AND CONCLUSION

1. Various calculations for establishing a pattern of the eruptive sequence representing a norm for each sex have been made. These calculations included:

    1. The mean eruptive time in days for each tooth
    2. The standard deviation
    3. The standard error
    4. The standard error of the difference between the means

2. Using these statistics it has been possible to show that no significant difference exists between the mean eruptive time of the deciduous teeth of a random group of boys and girls with the exception of one tooth, the upper left central incisor. Boys erupt this tooth earlier than girls.

3. By means of similar calculations it has been possible to show that there is no significant difference between the eruptive time of corresponding teeth in either sex.

4. It has been shown further that there is a significant difference between the eruptive time of pairs of teeth. Thus it has been possible to establish a sequence of eruption.

5. The results obtained from the calculations for the coefficient of variability for the same random group of boys and girls reveal that the incisors are relatively more variable than the cuspids and molars in both sexes.

6. The absolute variability expressed in the standard deviation for the mean eruptive time for each of the two sexes has been tested for significant differences. The results show that the amount of variability in eruptive time between the sexes with the exception of one pair of teeth

is insignificant. In the eruption of the upper laterals only is the measure of spread about the mean eruptive time greater in the females than in the males.

7. Comparisons between the results derived from the Toronto sample and that of other investigators have been made.

8. Having established a pattern of the eruptive sequence representing the norm for each sex, the patterns of all the individuals making up the sample were compared and an objective method devised for determining regular and irregular patterns of the eruptive sequence. Regular and irregular patterns of eruption have been shown to be based on sequence and timing.

9. An objective method for determining harmonious and inharmonious relation of the dental arches, based on width has been discussed.

10. Three samples made up of (1) all 90 cases; (2) 61 unrelated individuals; (3) 49 cases, excluding suckers and individuals with special dietary problems, have been observed for type of eruptive pattern and relation of the dental arches. The amount of concordance and discordance between the two processes have been tested by means of chi-square tests. The P value was less than .01 in each case revealing that an association between the whole eruptive pattern and the relation of the arches is not one of chance.

11. A test of the whole sample of 90 cases revealed further that eruption of certain teeth is associated with growth in width of the arch in the region of these teeth. The P value was found to be between .01 and .05.

12. The pattern of the sequence of eruption has been shown to be very similar in MZ and DZ twins.

13. The mean eruptive time of a group of MZ twins and that of a group of DZ twins has been compared.

14. Similarity in eruptive time between left and right teeth of an individual has been shown to be almost the same as that between left and right teeth of pairs of MZ twins. This was not true of DZ twins.

15. The intra-class correlation for MZ twins has been estimated. It is 0.91 for MZ twins and 0.56 for DZ twins. The effect of heredity on the eruptive time of a tooth has been estimated as 75%.

16. The effect of sucking habits with respect to the width changes of the dental arches has been discussed. The importance of studying the effects of the habit in a large group of MZ twins has been pointed out.

The present investigation has contributed to our knowledge of eruption by means of a detailed study of the clinical phase of this process. By means of statistical analyses differences in the eruptive time of the deciduous teeth, calculated in days, for the sexes separated, have been analysed. Meaningful differences between the eruptive time of teeth have been identified and a sequence of eruption has thereby been established

This sequence, called the mean eruptive sequence, was used for determining patterns of the eruptive sequence in a group of individuals, made up of boys, girls singletons, sibs and MZ and DZ twins. The mean eruptive sequence was not only essential to this study, but because it is based on a large sample it should prove of value as a reliable norm for future research, and particularly so since the time here is calculated in days instead of months (compare Minot 1873; Boas 1927; Logan and Kronfeld 1936; Doering and Allen 1942 and many others) and for each tooth separately instead of corresponding teeth together. It should perhaps be pointed out too that because of the need of such data, two studies were started quite independently, the Toronto one and one at the Fels Institute. Robinow, Richards and Anderson (1942) of the latter institute have also contributed a table of the mean eruptive time in days for the sexes separated.

Many men have studied the growth and development of the deciduous dental arches, chief among these have been Lewis and Lehman (1929), Goldstein and Stanton (1940) and Cohen (1940). Their findings make it clear that between the ages of 2 - 5 years there is little change in the width of these parts, but commencing with the period between 5 and 6 years there follows a distinct period of acceleration in growth in width of the dental arches. The arches of boys have been shown to be larger than those of girls and a difference in shape has also been observed. The rate of growth between the sexes is a controversial point among writers, as is the concept of physiological mesial shifting.

Although the present investigation has involved attention to sex differences and hereditary factors in eruption, this was unfortunately impossible in observations of the dental arches because the sample was too small. But since differences between sexes and the effect of heredity in the growth of the arches have been carefully studied by other investigators, fresh considerations were made by the present writer.

Where comparison was possible results from the Toronto Study were found to be in agreement with those of other investigators, and variation, which is the keynote not only of eruption but also in growth in width of the dental arches was also observed. Despite all the variation which exists in the processes under observation, nevertheless, some contribution has been made. By discerning patterns not only in eruption but also in the relation of the arches the aim of this study has been achieved. In other words, a comparison of the patterns of the eruptive sequence with the growth in width of the dental arches has revealed that there is an interrelation between the two phenomena. The technique of using patterns in discerning types of growth is not a new one, but this is probably the first time they have been used as a means of comparison between eruptive pattern and growth in width of the dental arches of the deciduous dentition.

This study has shown further, that heredity plays an important part in the time taken for a tooth to erupt. Other researchists have pointed out that heredity also governs occlusion. Since the present work has shown that eruptive pattern and growth in width of the dental arch are related phenomena then it is logical to assume that heredity is a causal factor in this relation.

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The practical application of this investigation is in no way obscure. Parents and dentists alike are anxious to know what sort of development is taking place in the dental arches of their children and patients respectively. It is proposed that parents should be encouraged to keep accurate and complete records of the eruption of the deciduous teeth of their children, from which the dentist may extract a clue as to the sort of growth taking place. If the pattern of the eruptive sequence is regular there is a 75% chance of the arches showing a harmonious relation; 25% chance that there will be a disparity between them. If the eruptive pattern is regular there is 69% chance that the arches will lack harmony in their relation; 31% chance that they will show no disparity. The warning of impending disharmony manifest in the eruptive pattern may be checked by a series of casts. The procedure is a simple one, comparatively inexpensive and hence feasible for the general practitioner.

APPENDIX "P"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1952

Subject: Part 1- An investigation of the mechanism of repair of the supporting structures of the teeth; Part 2 - Fusospirochetal infection and pre-existing inflammation.

Grantee: Dr. C.H. Best

Project No. DR 22 Amount of Grant: $4200

SUMMARY OF WORK

A paper on the regeneration of supporting structure of the teeth of dogs following the creation of epithelialized infected periodontal pockets has been submitted for publication.

A study has been planned to permit a comparison of the processes involved in repair of the supporting structure and those involved in the growth of these tissues. Information regarding both processes is of definite interest to dentistry.

Forty-seven young rats were used for this study. The growth process was studied when it was accelerated, in the normal, and when inhibited. The growth process was accelerated by daily injections of growth hormone and inhibited by hypophysectomy. The rats were divided in seven treatment or control groups. At the completion of the study the jaws were removed and fixed in 10% formol saline. Approximately 600 paraffin sections were made of the teeth and supporting structure on one side of the mandible and stained with hematoxylin and eosin. Further details are given in the longer report.

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September, 1952

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Grantee: Dr. C.H. Best

Project No. DR 22 Amount of Grant: $4200

A paper on the work reported at the last meeting of the Committee has been submitted for publication.

The next phase of this investigation of the mechanism of repair of the supporting structures is a study of the growth process in these tissues. While information regarding the growth of these structures is of definite interest to dentistry, the primary purpose of this investigation is to throw further light on the fundamental problems in repair.

The growth process is studied when accelerated, in the normal, and when inhibited. It is accelerated in young rats by daily injections of growth hormone and inhibited by hypophysectomy. This also permits a study of the effect of these factors on the growth of the supporting structures. It seems probable that the factors that influence the physiologic mechanisms involved in growth would similarly affect those involved in repair.

Forty-seven young rats were used in this study. They were divided in 7 groups for treatment or controls. At the end of the study the jaws were removed and fixed in 10% formol saline. Approximately 600 paraffin sections were made of the molar teeth and supporting structures on one side of the mandible and stained by hematoxylin and eosin. It is expected that additional sections will be required.

H.I.G. - Hypophysectomized rats fed ad lib. and injected with 1 mg. of growth hormone per day for 28 days.

Animal	Initial Wt.	Final Wt.	Gain
HIG 13	112 gms.	206 gms.	94 gms.
" 14	120	182	62
" 15	108	166	58
" 16	100	198	98
" 17	104	170	66
" 18	108	180	72
Total	652	1102	450
Average	108	183	75

Group 2

P.F.H.I.G. - Normal rats pair-fed with H.I.G.

Animal	Initial Wt.	Final Wt.	Gain
PFHIG 13	112 gms.	196 gms.	84 gms.
" 14	120	180	60
" 15	108	178	70
" 16	100	148	48
" 17	102	170	68
" 18	109	130	21
Total	651	1002	351
Average	108	137	58

Group 3

G.H. - Normal rats pair-fed with P.F.G.H. for 28 days and received 1 mg. growth hormone per day for 28 days.

Animal	Initial Wt.	Final Wt.	Gain
GH 3	110 gms.	206 gms.	96 gms.
" 4	111	208	97
" 5	106	190	84
" 6	132	230	98
" 7	130	270	140
" 8	124	168	44
Total	713	1272	559
Average	119	212	93

Group 4

P.F.G.H. - Normal rats fed ad lib. used as controls for G.H. group.

Animal	Initial Wt.	Final Wt.	Gain
PFGH 3	112 gms.	173 gms.	61 gms.
" 4	100	208	108
" 5	102	194	92
" 6	132	260	128
" 7	130	235	105
" 8	120	178	58
Total	696	1248	552
Average	116	208	92

Group 5

C.A.D. - Normal rats fed ad lib. for 28 days.

Animal	Initial Wt.	Final Wt.	Gain
CAD 5	111 gms.	173 gms.	62 gms.
" 6	98	168	70
" 7	98	148	50
" 8	110	166	56
" 9	120	261	141
" 10	126	254	128
" 11	119	238	119
Total	782	1408	626
Average	111	201	89

Group 6

H.C. - Hypophysectomized rats fed ad lib. for 28 days

Animal	Initial Wt.	Final Wt.	Gain
HC 8	102	96	-6
HC 9	98	94	-4
HC 10	109	104	-5
HC 11	95	104	+9
HC 12	98	94	-4
HC 13	86	98	+12
HC 14	93	94	+1
HC 15	108	102	-6
Total	789	786	-3
Average	98.6	98.2	

Group 7

P.F.C.H.C. - series - pair-fed controls for H.C. series for 28 days.

Animal	Initial Wt.	Final Wt.	Gain
PFCHC 8	106	114	+8
" 9	96	126	+30
" 10	97	140	+43
" 11	95	112	+17
" 12	85	126	+41
" 13	85	112	+27
" 14	90	123	+33
" 15	106	118	+12
Total	760	971	211
Average	95	121	26.3

Group 5

C.A.D. - Normal rats fed ad lib. for 28 days.

Animal	Initial Wt.	Final Wt.	Gain
CAD 5	111 gms.	173 gms.	62 gms.
" 6	98	168	70
" 7	98	148	50
" 8	110	166	56
" 9	120	261	141
" 10	126	254	128
" 11	119	238	119
Total	782	1408	626
Average	111	201	89

Group 6

H.C. - Hypophysectomized rats fed ad lib. for 28 days.

Animal	Initial Wt.	Final Wt.	Gain
HC 8	102	96	-6
HC 9	98	94	-4
HC 10	109	104	-5
HC 11	95	104	+9
HC 12	98	94	-4
HC 13	86	98	+12
HC 14	93	94	+1
HC 15	108	102	-6
Total	789	786	-3
Average	98.6	98.2	

Group 7

P.P.H.C. - Parathyroidectomized rats fed controls for B.D. series for 28 days.

Animal	Initial Wt.	Final Wt.	Gain
PPCHC 8	106	114	+8
" 9	96	126	+30
" 10	97	140	+43
" 11	95	112	+17
" 12	85	126	+41
" 13	85	112	+27
" 14	90	123	+33
" 15	106	118	+12
Total	760	971	211
Average	95	121	26.3

APPENDIX "Q"

Suggested Research Projects

By decision of the Committee (Min.4(x), 13th Mtg). projects suggested as research topics are listed hereunder for consideration by Committee members:

	Proposed Subject	Suggested by:	Date
1.	Gold inlays over amalgams Little information is available as to whether gold inlays over amalgams cause disintegration of the cement.	Dr. M.H. Garvin	October, 1950
2.	A study of root canals of teeth This is a very practical research subject as the results would be of value to every practitioner.	Dr. M.H. Garvin	October, 1950
3.	Social aspects of dental disease	Dr. A.C. Burton	September, 1951
4.	The effect of light upon foodstuffs in relation to dentistry.	Dr. M.H. Garvin	March, 1952
5.	To test adhesive qualities of acrylic filling material to tooth structure.	Dr. M.H. Garvin	September, 1952

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1. Gold inlays over amalgams Little information is available as to whether gold inlays over amalgams cause disintegration of the cement.	Dr. M.H. Garvin	October, 1950
2. A study of root canals of teeth This is a very practical research subject as the results would be of value to every practitioner.	Dr. M.H. Garvin	October, 1950
3. Social aspects of dental disease	Dr. A.C. Burton	September, 1951
4. The effect of light upon foodstuffs in relation to dentistry.	Dr. M.H. Garvin	March, 1952
5. To test adhesive qualities of acrylic filling material to tooth structure.	Dr. M.H. Garvin	September, 1952

APPENDIX "R"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

(i) List of Grantees and Subjects

nt No.	Grantee	Subject
1 ctive)	Dr. J.J. Rae, Dept. of Chemistry, University of Toronto	Chemical mechanisms in dental caries
2 ompleted)	Dr. G.H.W. Lucas, Dept. of Pharmacology, University of Toronto	A study of alveolar oxygen tension and blood oxygen content during nitrous oxide anaesthesia
3 ompleted)	Dr. H.K. Box, Faculty of Dentistry, University of Toronto	A study of micro-organisms found in deep periodontal pockets and caries lesion as revealed by the electron microscope
4 ompleted)	Dr. H.K. Box, Faculty of Dentistry, University of Toronto	An investigation of packing material in the treatment of periodontal disease (continuation of investigation previously carried on by Maj. Linghorne)
5 ompleted)	Major R.L. Twible, Royal Canadian Dental Corps, (later Ontario Research Foundation)	To improve certain properties of the acrylic resin denture base and to effect an economy in denture production
6 ompleted)	Col. E.M. Wansbrough, Royal Canadian Dental Corps	To study the effects of altitude acceleration and deceleration on oral tissues
7 ompleted)	Dr. J.S. Bagnall, Faculty of Dentistry, Dalhousie University	Critical survey of the literature on dental caries
8 ompleted)	Dr. A.D.A. Mason, Faculty of Dentistry, University of Toronto	A study of the D.M.F. caries rate of the children of Brantford prior to the addition of fluorine to the water supply
9 ompleted)	Dr. O.J. Walker, Dept. of Chemistry, Dr. H.R. MacLean, Faculty of Dentistry, University of Alberta	An investigation of the effects of certain chemicals on materials in appliances used in prosthetic dentistry
10 ompleted)	Dr. G.M. Macdonald, Queen Mary Veterans' Hospital, Montreal	Facial Prostheses

APPENDIX "K"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

(1) List of Grantees and Subjects

No.	Grantee	Subject
ive)	Dr. J.J. Rae, Dept. of Chemistry, University of Toronto	Chemical mechanisms in dental caries
leted)	Dr. G.H.W. Lucas, Dept. of Pharmacology, University of Toronto	A study of alveolar oxygen tension and blood oxygen content during nitrous oxide anaesthesia
leted)	Dr. H.K. Box, Faculty of Dentistry, University of Toronto	A study of micro-organisms found in deep periodontal pockets and caries lesion as revealed by the electron microscope
leted)	Dr. H.K. Box, Faculty of Dentistry, University of Toronto	An investigation of packing material in the treatment of periodontal disease (continuation of investigation previously carried on by Maj. Lingborne)
leted)	Major R.L. Twible, Royal Canadian Dental Corps, (later Ontario Research Foundation)	To improve certain properties of the acrylic resin denture base and to effect an economy in denture production
leted)	Col. E.M. Wansbrough, Royal Canadian Dental Corps	To study the effects of altitude acceleration and deceleration on oral tissues
leted)	Dr. J.S. Bagnall, Faculty of Dentistry, Dalhousie University	Critical survey of the literature on dental caries
leted)	Dr. A.D.A. Mason, Faculty of Dentistry, University of Toronto	A study of the D.M.F. caries rate of the children of Brantford prior to the addition of fluorine to the water supply
leted)	Dr. O.J. Walker, Dept. of Chemistry, Dr. H.R. Maclean, Faculty of Dentistry, University of Alberta	An investigation of the effects of certain chemicals on materials in applicances used in prosthetic dentistry
leted)	Dr. G.M. Macdonald, Queen Mary Veterans' Hospital, Montreal	Facial Prostheses

ant No.	Grantee	Subject
11 ompleted)	Dr. R.J. Godfrey, Faculty of Dentistry, University of Toronto	An anatomical, histological and physiological study of the role of the Condyle in Mastication
12 ompleted)	Dr. A.E.R. Westman, Ontario Research Foundation	Investigation of cements for methacrylate inlays
13 ctive)	Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Fluoride content of enamel and dentin of teeth from Toronto children studied in relation to pablum history of the children's diets
14 ompleted)	Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Fluorine analysis of water and food samples from different areas in Ontario collected and studied in relation to incidence of dental caries
15 ompleted)	Dr. Norma Ford Walker, Dept. of Zoology, University of Toronto	A comparison of the patterns of eruption of the deciduous teeth in man with rates of growth of the dental arches
16 ompleted)	Dr. C.H. Best, Dept. of Medical Research, University of Toronto	Studies in vitro of certain fungus-like organisms found in periodontal diseases
17 ompleted)	Dr. R.F. Shaner, Dept. of Anatomy, University of Alberta	An investigation of the effects of solutions used in operative dentistry on the vital pulps of teeth
18 ompleted)	Dr. H.K. Box, Faculty of Dentistry, University of Toronto	Experimental production of dental caries
19 ompleted)	Dr. E.A. Sellers, Dept. of Pharmacology, University of Toronto	Tissue toxicity and efficiency of local anaesthetics for topical application and nerve block in dentistry
20 ompleted)	Dr. Jules Thebaud, Faculty of Dental Surgery, University of Montreal	Improvement of sub-gingival curettage and surgical techniques used in the treatment of pyorrhea alveolaris

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2 (completed)	Dr. A.E.R. Westman, Ontario Research Foundation	Investigation of cements for methacrylate inlays
3 (active)	Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Fluoride content of enamel and dentin of teeth from Toronto children studied in relation to pedium history of the children's diets
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ant No.	Grantee	Subject
21 ompleted)	Dr. A.E.R. Westman, Ontario Research Foundation	Investigation of repair techniques for methacrylate dentures
22 ctive)	Dr. C.H. Best, Dept. of Medical Research, University of Toronto	Part I - An investigation of the mechanism of repair of the supporting structures of the teeth; Part 2 - Fusospirochetal infection and pre-existing inflammation
23 ctive)	Dr. C.P. Leblond, Dept. of Anatomy, McGill University	Entry of phosphate and carbonate salts into teeth as shown with the help of radio-phosphorus and radio-carbon
24 ompleted)	Dr. C.H.M. Williams, Faculty of Dentistry, University of Toronto	The effects of hard and soft diets on the supporting structures of the teeth
25 ompleted)	Dr. A.E.R. Westman, Ontario Research Foundation	Investigation of methods for the use of methyl methacrylate in direct dental fillings
26 ompleted)	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	A study of the normal and abnormal physiologic forces of the oral cavity
27 ompleted)	Dr. J.W. Abbiss Faculty of Dentistry, Dr. A.G. Nutlay, Faculty of Dentistry, Dalhousie University	A histological study of tooth germ development, both intra and extra alveolar, with a survey of pathological changes in the developing tooth
28 ctive)	Dr. O.J. Walker, Dept. of Chemistry, University of Alberta	Development of a method for determining fluorine by means of a photo-electric colorimeter
29 ompleted)	Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Biological absorption of fluorine
30 ompleted)	Dr. Norma Ford Walker, Dept. of Zoology, University of Toronto	The study of the closure of space following premature loss of deciduous teeth

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Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Biological absorption of fluorine
Dr. Norma Ford Walker, Dept. of Zoology, University of Toronto	The study of the closure of space following premature loss of deciduous teeth

rant No.	Grantee	Subject
31 completed)	Dr. L.B. Jaques, Dept. of Physiology, Mr. G.J. Millar, Dept. of Physiology, University of Saskatchewan	An investigation of nerve block
32 completed)	Dr. A.W. Ham, Dept. of Anatomy, University of Toronto	Investigation of the effect of adrenal cortical hormone imbalances on the various connective tissue structures of the growing incisor teeth of animals
33 completed)	Dr. H.A. Hunter, Faculty of Dentistry, University of Toronto	A study of calcifying potential of calcium oleate on the dental pulp
34 completed)	Dr. A.D'Iorio, Dept. of Physiology, University of Montreal	Studies of calcium metabolism in tooth with the aid of Ca^{45}
35 active)	Dr. J.B. Macdonald, Dept. of Bacteriology, University of Toronto	Bacteriology of periodontal disease. -I. Experimental fusospirochetal infection with mixtures of pure cultures
36 active)	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	Demineralization of hard as bone and teeth by organic chelating agents
37 active)	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	Further studies in the electro-myography of the head, face and neck
38 active)	Dr. K.J. Paynter, Faculty of Dentistry, University of Toronto	The effect of thiouracil on the development of molar teeth of rats
39 active)	Dr. A.G. Racey, Faculty of Dentistry, McGill University	Sectioning of teeth and surrounding structures and the effects of potassium deficiency and of pyriodoxine deficiency on the teeth and surrounding structures of dogs
40 completed)	Dr. L.A. Funt, Faculty of Dentistry, University of Toronto	Effect of tooth eruption and function on the growth of the mandible and facial complex on cats

Grant No.	Grantee	Subject
31 (completed)	Dr. L.B. Jaques, Dept. of Physiology, Mr. C.J. Millar, Dept. of Physiology, University of Saskatchewan	An investigation of nerve block
32 (completed)	Dr. A.W. Ham, Dept. of Anatomy, University of Toronto	Investigation of the effect of adrenal cortical hormone imbalances on the various connective tissue structure of the growing incisor teeth of animals
33 (completed)	Dr. H.A. Hunter, Faculty of Dentistry, University of Toronto	A study of salivation potential of calcium oleate on the dental pulp
34 (completed)	Dr. A.D'Iorio, Dept. of Physiology, University of Montreal	Studies of calcium metabolism in tooth with the aid of Ca45
35 (active)	Dr. J.D. Macdonald, Dept. of Bacteriology, University of Toronto	Bacteriology of periodontal disease. -I. Experimental fusospirochetal infection with mixtures of pure cultures
36 (active)	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	Demineralization of hard as bone and teeth by organic chelating agents
37 (active)	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	Further studies in the electro-myography of the head, face and neck
38 (active)	Dr. K.J. Paynter, Faculty of Dentistry, University of Toronto	The effect of thiouracil on the development of molar teeth of rats
39 (active)	Dr. A.G. Racey, Faculty of Dentistry, McGill University	Sectioning of teeth and surrounding structures and the effects of potassium deficiency and of pyridoxine deficiency on the teeth and surrounding structures of dogs
40 (completed)	Dr. L.A. Punt, Faculty of Dentistry, University of Toronto	Effect of tooth eruption and function on the growth of the mandible and facial complex on cats

rant No.	Grantee	Subject
R 41 completed)	Dr. Harvey Jenkins, Faculty of Dentistry, University of Toronto	A study of the dental arch form and certain of its anatomical relations in a series of clinically excellent dentitions (After Downs(1))
R 42 active)	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	Studies in the chemistry of the saliva. II. The reducing property of saliva towards some oxidation-reduction indicators
R 43 active)	Dr. S.G. Davis, Dept. of Chemistry, Dr. H.R. MacLean, Faculty of Dentistry, University of Alberta	Electro potentials between dental alloys
R 44 active)	Dr. Arthur Harry Craven, Faculty of Dentistry, McGill University	The growth and width of the face and head of the Macaque monkey as revealed by vital staining
R 45 active)	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	Study of the incidence of malocclusion
R 46 active)	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	An analysis of treated orthodontic cases
R 47 active)	Dr. H.A. Hunter, Faculty of Dentistry, University of Toronto	The pathological characteristics of experimental fusopirochaetal infections

Grant No.	Grantee	Subject
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42 (active)	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	Studies in the chemistry of the saliva. II. The reducing property of saliva towards some oxidation-reduction indicators
43 (active)	Dr. S.G. Davis, Dept. of Chemistry, Dr. H.R. MacLean, Faculty of Dentistry, University of Alberta	Electro potentials between [illegible] alloys
44 (active)	Dr. Arthur Henry Graven, Faculty of Dentistry, McGill University	The growth and width of the face and head of the Macaque monkey as revealed by vital staining
45 (active)	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	Study of the incidence of malocclusion
46 (active)	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	An analysis of treated orthodontic cases
47 (active)	Dr. H.A. Hunter, Faculty of Dentistry, University of Toronto	The pathological characteristics of experimental fusopirochaetal infections

APPENDIX "R"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

(ii) Summary of Grants by Year 1945-53

Grant No.	Grantee	1945-46 $	1946-47 $	1947-48 $	1948-49 $	1949-50 $	1950-51 $	1951-52 $	1952-53 $
DR 1	Rae	1000	900	1000 250	1760 150	1000 550	950	960	1530
DR 2	Lucas	1550	2950	1900	1200 400	325	1550	-	-
DR 3	Box	1000	500	500	300	100	-	-	-
DR 4	Linghorne R.C.D.C.	-	(Box) 2200 1000	2200	-	-	-	-	-
DR 5	Twible R.C.D.C.	-	(Ont.Res. Foundation) 4800	-	-	-	-	-	-
DR 6	Coons R.C.D.C.	-	Wansbrough	-	-	-	-	-	-
DR 7	Bagnall	200	300	225	-	75	-	-	-
DR 8	Ellis (Mason)	-	1000	completed	-	-	-	-	-
DR 9	Walker MacLean	-	1000	1000	1100	300	-	-	-
DR 10	Macdonald	-	800	completed	-	-	-	-	-
DR 11	Godfrey	-	1425 (half-year)	2550	2200	559.07	-	-	-
DR 12	Westman	-	-	2550	See DR 21	See DR 25	See DR25	See DR25	-

APPENDIX "B"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

(111) Summary of Grants by Year 1974-75

Grant No.	Grantee	1974-75 $	1975-76 $	1976-77 $	1977-78 $	1978-79 $	1979-80 $	1980-81 $	1981-82 $
DR 1	Rae	1000	900	1000	1000	1000	950	900	1250
DR 2	Lucas	1250	1250	1000	950	1000	1250	-	-
DR 3	Box	1000	500	500	500	300	-	-	-
DR 4	Livingstone	-	-	2000 (Box)	-	-	-	-	-
DR 5	Table R.C.D.C.	-	-	2000 (Ont. Res. Foundation)	-	-	-	-	-
DR 6	Coons R.C.D.C.	-	-	Wansbrough 1800	-	-	-	-	-
DR 7	Bagnall	200	300	500	225	-	75	-	-
DR 8	Ellis (Mason)	-	1000	1000	completed	-	-	-	-
DR 9	Walker Maclean	-	-	1000	1000	1000	500	-	-
DR 10	Macdonald	-	800	-	completed	-	-	-	-
DR 11	Godfrey	-	1215 (Dalhousie)	1500	1250	750	550	-	-
DR 12	Wessman	-	-	-	-	-	-	-	-

- 1250 see DR 12 see DR 25 see DR25

Grant No.	Grantee	1945-46 $	1946-47 $	1947-48 $	1948-49 $	1949-50 $	1950-51 $	1951-52 $	1952-53 $
DR 13	Smith	-	-	1050	1455	1475	1475	1600 75	500
DR 14	Smith	-	-	1125	1500	-	-	-	-
DR 15	Walker N.F.	-	-	3000	3000	2500	800	-	-
DR 16	Best	-	-	1100	1500	-	-	-	-
DR 17	Shaner	-	-	1000	-	-	-	-	-
DR 18	Box	-	-	1350	1620	1620 458.80	-	-	-
DR 19	Sellers	-	-	1300	1575	Ferguson 350	-	-	-
DR 20	Thebaud	-	-	800	750	750	-	-	-
DR 21	Westman	-	-	-	5000	-	-	-	-
DR 22	Box (later Best)	-	-	-	2200	2400	3800	2300 875 1350	4200
DR 23	Leblond	-	-	-	2200	2200 400	2400	2400	3000
DR 24	Williams	-	-	-	1420	3080	1735	-	-
DR 25	Westman (Ontario Res.Foundation)	-	-	-	-	4000	3000	4000	-
DR 26	Moyers	-	-	-	-	1300	150	-	-

Grant No.	Grantee	70-71	71-72	72-73	73-74	74-75	75-76	76-77	77-78	78-79	79-80
DR 53	Smith	–	–	–	–	–	–	–	–	–	–
DR 54	Smith	–	–	–	–	–	–	–	–	–	–
DR 55	Walker	–	–	–	–	–	–	800	2500	3000	3000
DR 56	Beat	–	–	–	–	–	–	–	–	–	–
DR 57	Shaver	–	–	–	–	–	–	–	–	–	–
DR 58	Box	–	–	–	1230	1650	2050	2500	2500	2500	2500
DR 59	Sellers	–	–	–	1230	1575	Personnel 250	–	–	–	–
DR 60	Thurgood	–	–	800	750	750	–	–	–	–	–
DR 61	Weekman	–	–	–	500	–	–	–	–	–	–
DR 62	Box (ret.) Beat()	–	–	–	2200	2200	2500	2500	2500	2500	2500
DR 63	Redford	–	–	–	2200	2200	2500	2500	2500	2500	2500
DR 64	Williams	–	–	–	1750	3080	400	2500	2500	2500	2500
DR 65	Weekman (Ortero) Beat(Boundaries)	–	–	–	1000	1000	3000	3000	3000	3000	3000
DR 66	Moyers	–	–	–	1500	1500	1750	2500	2500	2500	2500

Grant No.	Grantee	1945-46 $	1946-47 $	1947-48 $	1948-49 $	1949-50 $	1950-51 $	1951-52 $	1952-53 $
DR 27	Abbiss Nutlay	-	-	-	-	1000	200	-	-
DR 28	Walker O.J. MacLean	-	-	-	-	1000	1000	1000	600
DR 29	Smith	-	-	-	-	1500	375	-	-
DR 30	Walker N.F.	-	-	-	-	1200 600	1400 1200	-	-
DR 31	Jaques Millar	-	-	-	-	-	1870	188	-
DR 32	Ham	-	-	-	-	-	900	-	-
DR 33	Hunter	-	-	-	-	-	1500	1600	-
DR 34	D'Iorio	-	-	-	-	-	2840	1200 (Lussier)	-
DR 35	Macdonald	-	-	-	-	-	2000	2800	3300
DR 36	Nikiforuk	-	-	-	-	-	-	2400 350	1600
DR 37	Moyers	-	-	-	-	-	-	960	800
DR 38	Paynter	-	-	-	-	-	-	1850	2610
DR 39	Racey	-	-	-	-	-	-	1600	1600

Cust No.	Customer	1st-15th	15-30th	1st-15th	15-30th	1st-15th	15-30th	1st-15th	15-30th
DR 27	Abbies Bakery	-	-	-	-	-	-	-	-
DR 28	Walker O.J. Masters	-	-	-	-	1000	1000	600	-
DR 29	Smith	-	-	-	-	575	-	-	-
DR 30	Walker N.F.	-	-	-	-	1000	1000	-	-
DR 31	Jones Miller	-	-	-	-	1000	900	-	-
DR 32	Ham	-	-	-	-	909	-	-	-
DR 33	Hunter	-	-	-	-	1500	1000	-	-
DR 34	D'Iorio	-	-	-	-	2840	1200 (Travel)	-	-
DR 35	Macdonald	-	-	-	-	2000	2800	3200	-
DR 36	Whitlock	-	-	-	-	-	2400	1600	-
DR 37	Noyes	-	-	-	-	-	650	800	-
DR 38	Peyton	-	-	-	-	-	1850	2120	-
DR 39	Kacy	-	-	-	-	-	-	1000	-

Grant No.	Grantee	1945-46 $	1946-47 $	1947-48 $	1948-49 $	1949-50 $	1950-51 $	1951-52 $	1952-53 $
DR 40	Funt Sayers	-	-	-	-	-	-	325	-
DR 41	Jenkins	-	-	-	-	-	-	300	-
DR 42	Nikiforuk	-	-	-	-	-	-	-	1500
DR 43	Davis MacLean	-	-	-	-	-	-	-	150
DR 44	Craven	-	-	-	-	-	-	-	600
DR 45	Moyers	-	-	-	-	-	-	-	1700
DR 46	Moyers	-	-	-	-	-	-	-	520
DR 47	Hunter	-	-	-	-	-	-	-	1200

Grant No.	Grantee	1945-46 $	1946-47 $	1947-48 $	1948-49 $	1949-50 $	1950-51 $	1951-52 $	1952-53 $
DR 40	Pumphrey Sayers	-	-	-	-	-	-	252	-
DR 41	Jenkins	-	-	-	-	-	-	300	-
DR 42	Mihajlovich	-	-	-	-	-	-	-	1500
DR 43	Davis McClean	-	-	-	-	-	-	-	150
DR 44	Craven	-	-	-	-	-	-	-	600
DR 45	Moyers	-	-	-	-	-	-	-	1700
DR 46	Moyers	-	-	-	-	-	-	-	550
DR 47	Humphrey	-	-	-	-	-	-	-	1200

Members of the Associate Committee on Dental Research and its Executive, with their terms of appointment

		Term to Expire
*1.	Dr. R.G. Ellis, Chairman, Dean, Faculty of Dentistry, University of Toronto, Toronto, Ont.	31 March, 1954
2.	Dr. E.W.R. Steacie, ex officio, President, National Research Council, Ottawa, Ont.	---
	Representing the Dental Profession	
3.	Dr. J.S. Bagnall, Dean, Faculty of Dentistry, Dalhousie University, Halifax, N.S.	31 March, 1955
*4.	Dr. E. Charron, Dean of the Faculty of Dental Surgery, University of Montreal, Montreal, Que.	31 March, 1954
5.	Dr. M.H. Garvin, Editor-in-Chief, Journal of the Canadian Dental Assoc., 314 Somerset Building, Winnipeg, Man.	31 March, 1953
6.	Dr. H.R. MacLean, Lecturer in Operative Dentistry, University of Alberta, Edmonton, Alta.	31 March, 1953
7.	Dr. R.H. McDougall, 1505 Hampshire Road, Victoria, B.C.	31 March, 1955
	Representing the Royal Canadian Dental Corps	
*8.	Brig. E.M. Wansbrough, ex officio Director General of Dental Services, Royal Canadian Dental Corps, Dept. of National Defence, Ottawa, Ont.	---
	Representing the Division of Dental Health Department of National Health and Welfare	
9.	Dr. H.K. Brown, ex officio, Chief, Dental Health Division, Dept. of National Health and Welfare, Ottawa, Ont.	---

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Representing the Dental Profession

3.	Dr. L.S. Beanall, Dean, Faculty of Dentistry, Dalhousie University, Halifax, N.S.	31 March, 1955
#4.	Dr. E. Charron, Dean of the Faculty of Dental Surgery, University of Montreal, Montreal, Que.	31 March, 1954
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6.	Dr. H.R. Maclean, Lecturer in Operative Dentistry, University of Alberta, Edmonton, Alta.	31 March, 1953
7.	Dr. R.H. McDougall, 1505 Hampshire Road, Victoria, B.C.	31 March, 1955

Representing the Royal Canadian Dental Corps

#8.	Brig. E.M. Wansbrough, ex officio Director General of Dental Services, Royal Canadian Dental Corps, Dept. of National Defence, Ottawa, Ont.	---

Representing the Division of Dental Health Department of National Health and Welfare

9.	Dr. H.K. Brown, ex officio, Chief, Dental Health Division, Dept. of National Health and Welfare, Ottawa, Ont.	---

Representing the Dental Research, Department of Veterans' Affairs

10. Dr. L.A. Kilburn, ex officio — ---
Director of Dental Research,
Dept. of Veterans' Affairs,
Ottawa, Ont.

Representing the Basic and Medical Sciences

11. Dr. C.H. Best, — 31 March, 1953
Director, Banting and Best Dept. of
Medical Research,
University of Toronto,
Toronto, Ont.

12. Dr. A.C. Burton, — 31 March, 1954
Head of the Dept. of Biophysics,
University of Western Ontario,
London, Ont.

13. Dr. A.W. Ham, — 31 March, 1955
Professor of Anatomy,
Faculty of Medicine,
University of Toronto,
Toronto, Ont.

14. Dr. J.D. Hamilton, — 31 March, 1954
Professor of Pathology,
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15. Dr. J.B. Macdonald, — 31 March, 1954
Assistant Professor of Bacteriology,
Faculty of Dentistry,
University of Toronto,
Toronto, Ont.

16. Dr. Edouard Pagé, — 31 March, 1955
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Department of Nutrition,
Medical School,
Laval University,
Quebec, Que.

17. Dr. J.J. Rae, — 31 March, 1953
Dept. of Chemistry,
University of Toronto,
Toronto, Ont.

x18. Dr. D.L. Thomson, — 31 March, 1953
Chairman of the Dept. of Biochemistry,
McGill University,
Montreal, Que .

Representing the Dental Research,
Department of Veterans' Affairs

10. Dr. L.A. Kilburn, ex officio ---
Director of Dental Research,
Dept. of Veterans' Affairs,
Ottawa, Ont.

Representing the Basic and Medical Sciences

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Public Relations Branch,
National Research Council,
Ottawa, Ont.

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x Members of the Executive

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21. McGill - Dr. D. Prescott Mowry

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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS

OF THE

ELEVENTH MEETING

OF THE

EXECUTIVE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

OTTAWA

2 MARCH 1953

NATIONAL RESEARCH COUNCIL

Proceedings

Eleventh Meeting

of the

EXECUTIVE

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in Room 427, Chateau Laurier, 8 p.m., Monday, 2 March, 1953.

1. Attendance

Dr. R.G Ellis, Chairman
Dr. E. Charron
Dr. D.L. Thomson
Brig. E.M. Wansbrough

Mr. S.J. Cook, Secretary

Others present were:

Dr. J.S. Bagnall
Dr. J.B. Macdonald
Dr. H.A. Hunter

2. Applications for Grants

The Executive reviewed all applications for grants including renewals and new applications and made the following recommendations:

(i) Grant No.	Grantee	Amount	Action
DR 1	Dr. J.J. Rae	$1680	Recommended
DR 22	Dr. C.H. Best	$4200	Recommended

The Executive was of the opinion that the continued employment of any one scientist under a research grant was detrimental to his own interest, and that this particular project was now sufficiently well established to permit the withdrawal of the Committee's support at the end of this present year.

Grant No.	Grantee	Amount $	Action
DR 23	Dr. C.P. Leblond	3000	Recommended
DR 28	Dr. O.J. Walker	1000	"
DR 35	Dr. J.B. Macdonald	3610	"
DR 37	Dr. R.E. Moyers	300	"
DR 38	Dr. K.J. Paynter	1000	"
	This project should be completed this year.		
DR 43	Dr. H.R. MacLean and Dr. S.G. Davis	1025	Recommended
	Pilot study to be completed this year.		
DR 44	Dr. A.H. Craven	1350	Recommended
DR 46	Dr. R.E. Moyers	520	"
DR 47	Dr. H.A. Hunter	2200	"

(ii) New Applications

1.	Dr. G. Nikiforuk	2240	(Set up as new project)
	Application for $2740 was reduced by $500, deleting Section 2, for animals and their upkeep.		Recommended
2.	Dr. A.H. Craven	400	Recommended
3.	Dr. J.B. Macdonald	400	"
4.	Dr. R.L. Saunders	1200	"
5.	Dr. C.B. Weld	4760	"
6.	Dr. M. Doreen Smith	2600	Not Recommended
	The Executive suggested that favourable consideration be given to an application for a renewal under DR 13.		
7.	Dr. E.E. Johns	600	Not Recommended
	(Application was for travel funds exclusively; chairman and Executive to act, under Committee travel allowance).		

(iii) Summary

Renewals	$19,885
New Applications	9,000
TOTAL	$28,785

Grant No.	Grantee	Amount	Action
		$	
DR 23	Dr. C.F. Lebland	3000	Recommended
DR 28	Dr. O.J. Wilder	1000	"
DR 35	Dr. J.B. Macdonald	3610	"
DR 37	Dr. R.E. Moyers	700	"
DR 38	Dr. K.J. Paynter	1000	"
			Trois projects should be completed this year.
DR 43	Dr. H.K. Maclean		
	and		
	Dr. S.G. Davis	1025	Recommended
			Pilot study to be completed this year.
DR 44	Dr. A.H. Craven	1350	Recommended
DR 45	Dr. R.E. Moyers	520	"
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(iii) Summary

Renewals	$19,885
New Applications	9,000
TOTAL	$28,785

3. Membership of the Committee

THE CHAIRMAN pointed out that five members, having completed two terms each, would be ineligible for re-appointment for at least one year, and he asked for nominations to replace them. There would be two vacancies for dentists, and three for representatives of the basic sciences, to maintain the Committee establishment on the authorized basis.

Numerous names were suggested and it was agreed to submit the following:-

Dentists:-

DR. C.D. HUSBAND, Red Deer, Alberta

DR. A.G. RACEY, McGill University

Basic Sciences:-

DR. J. ALDOUS, Head of the Department of Pharmacology,
Dalhousie University,
Halifax, N.S.

DR. J.M. BEVERIDGE, Head, Department of Biochemistry,
Queen's University,
Kingston, Ont.

DR. R.E. HAIST, Professor of Physiology,
University of Toronto,
Toronto, Ont.

DR. M.M. HOFFMAN, Department of Medicine,
McGill University

4. Date of Next Meeting

It was AGREED to recommend that the next meeting of the Committee be held in Montreal, Thursday, 22 October, 1953, immediately following the meetings there of the Canadian Dental Association.

Dr. Charron said he would be happy to arrange for the accommodation of the Committee in the School of Dentistry, University of Montreal, on that occasion.

The Meeting adjourned at 10 P.M.

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DR. C.D. HUSBAND, Red Deer, Alberta

DR. A.G. MACEY, McGill University

Basic Sciences:-

DR. J. ALDOUS, Head of the Department of Pharmacology, Dalhousie University, Halifax, N.S.

DR. J.M. BRYDRIDGE, Head, Department of Biochemistry, Queen's University, Kingston, Ont.

DR. R.E. HALST, Professor of Physiology, University of Toronto, Toronto, Ont.

DR. M.M. HOFFMAN, Department of Medicine, McGill University

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The Meeting adjourned at 10 P.M.

Members of the Associate Committee on Dental Research

and its Executive, with their terms of appointment

		Term to Expire
x1.	Dr. R.G. Ellis, Chairman, Dean of the Faculty of Dentistry, University of Toronto, Toronto, Ont.	31 March, 1954
2.	Dr. E.W.R. Steacie, ex officio, President, National Research Council, Ottawa, Ont.	---
	Representing the Dental Profession	
3.	Dr. J.S. Bagnall, Dean, Faculty of Dentistry, Dalhousie University, Halifax, N.S.	31 March, 1955
x4.	Dr. E. Charron, Dean of the Faculty of Dental Surgery, University of Montreal, Montreal, Que.	31 March, 1954
5.	Dr. M.H. Garvin, Editor-in-Chief, Journal of the Canadian Dental Assoc., 314 Somerset Building, Winnipeg, Man.	31 March, 1953
6.	Dr. H.R. MacLean, Lecturer in Operative Dentistry, University of Alberta, Edmonton, Alta.	31 March, 1953
7.	Dr. R.H. McDougall, 1505 Hampshire Road, Victoria, B.C.	31 March, 1955
	Representing the Royal Canadian Dental Corps	
x8.	Brig. E.M. Wansbrough, ex officio Director General of Dental Services, Royal Canadian Dental Corps, Dept. of National Defence, Ottawa, Ont.	---
	Representing the Division of Dental Health Department of National Health and Welfare	
9.	Dr. H.K. Brown, ex officio, Chief, Dental Health Division, Dept. of National Health and Welfare, Ottawa, Ont.	---

Members of the Associate Committee on Dental Research
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Term to Expire

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Dean of the Faculty of Dentistry,
University of Toronto,
Toronto, Ont.

2. Dr. E.W.R. Steacie, ex officio, ---
President,
National Research Council,
Ottawa, Ont.

Representing the Dental Profession

3. Dr. J.S. Bagnall, 31 March, 1955
Dean, Faculty of Dentistry,
Dalhousie University;
Halifax, N.S.

*4. Dr. E. Cherron, 31 March, 1954
Dean of the Faculty of Dental Surgery,
University of Montreal,
Montreal, Que.

5. Dr. M.H. Garvin, 31 March, 1953
Editor-in-Chief,
Journal of the Canadian Dental Assoc.,
314 Somerset Building,
Winnipeg, Man.

6. Dr. H.R. MacLean, 31 March, 1953
Lecturer in Operative Dentistry,
University of Alberta,
Edmonton, Alta.

7. Dr. E.H. McDougall, 31 March, 1955
1505 Hampshire Road,
Victoria, B.C.

Representing the Royal Canadian Dental Corps

*8. Brig. E.M. Wansbrough, ex officio ---
Director General of Dental Services,
Royal Canadian Dental Corps,
Dept. of National Defence,
Ottawa, Ont.

Representing the Division of Dental Health
Department of National Health and Welfare

9. Dr. H.E. Brown, ex officio, ---
Chief, Dental Health Division,
Dept. of National Health and Welfare,
Ottawa, Ont.

Representing the Dental Research,
Department of Veterans' Affairs

10.	Dr. L.A. Kilburn, ex officio Director of Dental Research, Dept. of Veterans' Affairs, Ottawa, Ont.	---

Representing the Basic and Medical Sciences

11.	Dr. C.H. Best, Director, Banting and Best Dept. of Medical Research, University of Toronto, Toronto, Ont.	31 March, 1953
12.	Dr. A.C. Burton, Head of the Dept. of Biophysics, University of Western Ontario, London, Ont.	31 March, 1954
13.	Dr. A.W. Ham, Professor of Anatomy, Faculty of Medicine, University of Toronto, Toronto, Ont.	31 March, 1955
14.	Dr. J.D. Hamilton, Professor of Pathology, University of Toronto, Toronto, Ont.	31 March, 1954
15.	Dr. J.B. Macdonald, Assistant Professor of Bacteriology, Faculty of Dentistry, University of Toronto, Toronto, Ont.	31 March, 1954
16.	Dr. Edouard Page, Director, Department of Nutrition, Medical School, Laval University, Quebec, Que.	31 March, 1955
17.	Dr. J.J. Rae, Dept. of Chemistry, University of Toronto, Toronto, Ont.	31 March, 1953
x18.	Dr. D.L. Thomson, Chairman of the Dept. of Biochemistry, McGill University, Montreal, Que.	31 March, 1953

Representing the Dental Research,
Department of Veterans' Affairs

10. Dr. L.A. Kilburn, ex officio ---
Director of Dental Research,
Dept. of Veterans' Affairs,
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Public Relations Branch,
National Research Council,
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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS

OF THE

SEVENTEENTH MEETING

OF THE

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

OTTAWA

2 MARCH 1953

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APPENDICES

Appendix "A" Papers Published, Associate Committee on Dental Research

Appendix "B" Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radio-active elements, DR 23

Appendix "C" The effect of the endocrines on the teeth and jaws, DR 38

Appendix "D" Experimental fusospirochetal infection with mixtures of pure cultures, DR 35

Appendix "E" Chemical mechanism in dental caries, DR 1

Appendix "F" A histopathological study of the effects of a potassium deficiency and of a pyridoxin deficiency on the teeth and surrounding structures of dogs, DR 39

Appendix "G" An investigation of the mechanism of repair of the supporting structures of the teeth, DR 22

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Appendix "I" The growth in width of the face and head of the Macaque monkey as revealed by vital staining, DR 44

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Appendix "K"	An analysis of treated orthodontic cases, DR 46
Appendix "L"	Studies in the chemistry of the saliva, DR 42
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Appendix "S"	List of grantees and subjects
Appendix "T"	Importance of fluoridation as a public health measure by J.C. Bouillon and Fluoridation and the health officer by Ad. Groulx.

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NATIONAL RESEARCH COUNCIL

Proceedings

of the

Seventeenth Meeting

of the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in Council Chamber, National Research Building,
9:30 a.m.,Monday, 2 March, 1953.

---

1. Attendance

Dr. R.G. Ellis, Chairman
Dr. E.W.R. Steacie, President NRC
Dr. J.S. Bagnall
Dr. H.K. Brown
Dr. A.C. Burton
Dr. E. Charron
Dr. M.H. Garvin
Dr. A.W. Ham
Dr. J.D. Hamilton
Dr. L.A. Kilburn
Dr. J.B. Macdonald
Dr. H.R. MacLean
Dr. R.H. McDougall
Dr. E. Pagé
Dr. J.J. Rae
Dr. D.L. Thomson
Brig. E.M. Wansbrough

Mr. S.J. Cook, Secretary

By invitation

Dr. H.A. Hunter
Dr. M. Doreen Smith

2. Chairman's Remarks

THE CHAIRMAN expressed gratification at the good representation of members attending this meeting and welcomed especially Dr. M. Doreen Smith and Dr. H.A. Hunter, grantees, who had come to report on their respective projects.

NATIONAL RESEARCH COUNCIL

Proceedings

of the

Seventeenth Meeting

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ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in Council Chamber, National Research Building,
9:30 a.m., Monday, 2 March, 1953.

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Dr. J.D. Hamilton
Dr. I.A. Kilburn
Dr. J.B. Macdonald
Dr. H.R. Maclean
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Brig. E.M. Wansbrough

Mr. S.J. Cook, Secretary

By invitation

Dr. H.A. Hunter
Dr. M. Doreen Smith

2. Chairman's Remarks

THE CHAIRMAN expressed gratification at the good representation of members attending this meeting and welcomed especially Dr. M. Doreen Smith and Dr. H.A. Hunter, grantees, who had come to report on their respective projects.

3. Minutes of the 16th Meeting

It was MOVED by Dr. Bagnall and SECONDED by Brig. Wansbrough,

That the minutes of the 16th meeting, having been circulated, be taken as read and approved. CARRIED.

4. Business Arising Out of the Minutes

(i) Work with Radioactive Elements (Min.8, 16th Mtg.)

THE CHAIRMAN said that it had been suggested by Dr. Burton that Dr. Bélanger might be encouraged to seek the assistance of the Committee in his work since he was interested in this particular field. No action however had been taken as yet to communicate with Dr. Bélanger. (An application for a grant-in-aid was subsequently received from him).

(ii) Meetings of the Committee (Min. 18, 16th Mtg.)

The question as to whether the Committee should continue to hold two meetings a year is to be reviewed at this meeting.

(iii) Dr. Bagnall's Bibliography (Min. 4(i), 16th Mtg.)

THE SECRETARY reported that 232 copies of the Bibliography had been distributed up to March 1953, including 37 complimentary copies and 195 copies disposed of by sale. This leaves 68 copies still on hand as compared with 80 copies in March 1952.

(iv) A Survey of the Literature of Dental Caries

THE SECRETARY reported that he had received, in exchange for a copy of Dr. Bagnall's Bibliography, a volume under the above title prepared for the food and nutrition board of the National Research Council with the supervision of the Committee on Dental Health of the National Research Council, Washington, D.C. It was issued as publication 225 of the Council and was dated 1952. It is a volume of 567 pages including a bibliography of about 2000 references. No mention was made in this bibliography of Dr. Bagnall's monumental work.

Consideration of Reports from Grantees

5. DR 1 - Dr. J.J. Rae

"Chemical Mechanisms in Dental Caries"

DR. RAE expressed his thanks to the Committee for having provided him with travel funds which permitted him to attend the December 1952 meeting of the American Association for the Advancement of Science at which he had given a paper on "Ammonia Production in Saliva". He said that he had included a summary of his address

3. Minutes of the 165th Meeting

It was MOVED by Dr. Bagnall and SECONDED by Brig. Wansbrough,

That the minutes of the 165th meeting, having been circulated, be taken as read and approved. CARRIED.

4. Business Arising Out of the Minutes

(i) Work with Radioactive Elements (Min.3, 165th Mtg.)

THE CHAIRMAN said that it had been suggested by Dr. Burton that Dr. Belanger might be encouraged to seek the assistance of the Committee in his work since he was interested in this particular field. No action however had been taken as yet to communicate with Dr. Belanger. (An application for a grant-in-aid was subsequently received from him).

(ii) Meetings of the Committee (Min. 18, 165th Mtg.)

The question as to whether the Committee should continue to hold two meetings a year is to be reviewed at this meeting.

(iii) Dr. Bagnall's Bibliography (Min. 4(i), 165th Mtg.)

THE SECRETARY reported that 232 copies of the Bibliography had been distributed up to March 1953, including 37 complimentary copies and 195 copies disposed of by sale. This leaves 68 copies still on hand as compared with 80 copies in March 1952.

(iv) A Survey of the Literature of Dental Caries

THE SECRETARY reported that he had received, in exchange for a copy of Dr. Bagnall's Bibliography, a volume under the above title prepared for the Food and Nutrition board of the National Research Council with the supervision of the Committee on Dental Health of the National Research Council, Washington, D.C. It was issued as publication 225 of the Council and was dated 1952. It is a volume of 567 pages including a bibliography of about 2000 references. No mention was made in this bibliography of Dr. Bagnall's monumental work.

Consideration of Reports from Grantees

5. DR 1 - Dr. J.J. Rae

"Chemical Mechanisms in Dental Caries"

DR. RAE expressed his thanks to the Committee for having provided him with travel funds which permitted him to attend the December 1952 meeting of the American Association for the Advancement of Science at which he had given a paper on "Ammonia Production in Saliva". He said that he had included a summary of his address

for the information of the members as the last page of his interim report No. 15 which he would now present. The summary and complete report appear in APPENDIX "E"

6. DR 13 - Dr. M. Doreen Smith

"Fluoride Content of Enamel and Dentin of Teeth from Toronto Children studied in Relation to Pablum History of the Children's Diets"

THE CHAIRMAN said that Dr. Smith had been invited to present her report in person because this is a subject that is of very timely interest.

Dr. Smith presented a summary and her complete report both of which appear in APPENDIX "H"

DR. GARVIN said that a recent article in "Harpers" concludes that fluoridating a municipal water supply is not as effective in the prevention of caries as when fluorine occurs naturally in the drinking water supply. He would like to know something about the condition of the teeth in the Brantford area prior to June 1945 when that city began to treat its water supply by adding fluorine.

DR. SMITH replied that her samples of teeth did not go back that far. She thought it would be important to continue the analyses of the Brantford teeth for some time to come. There was no doubt that some of the teeth submitted to her for examination had been fully formed before the fluorine treatment of the city water supply had been undertaken.

DR. GARVIN inquired as to the form in which fluorine occurs in Pablum. Is it sodium or calcium?

DR. SMITH said sodium is more absorbable. The growing child has need of calcium and the amount absorbed depends on the proportion of phosphorus in the diet. There is some absorption of fluorine from the calcium salt, however, as well as from the sodium compound.

DR. BURTON questioned the wording of para. 2 on page "H-3" - "Apparently the soluble fluoride added to water increases the fluoride content of both dentin and enamel and apparently the dentin to a greater extent than the enamel" and after some discussion it was agreed to eliminate this paragraph from the report because it might be misleading to abstractors.

DR. HAM suggested that a further study should be made of Brantford teeth.

DR. BROWN said Dr. Smith has not yet had a chance to examine the teeth from Brantford, erupted since June 1945. He agreed that it was important to continue the study at this time.

THE CHAIRMAN said that the teeth selected for study should be taken from those formed since fluoridation was put into effect. Another experiment which might be worthwhile would be a study of the periodontal structures in animals. Little is known as to the effect of fluorine in the alveolar process.

DR. CHARRON drew the attention of the Committee to an article by Dr. J.C. Bouillon, Chief of the Section of Dental Hygiene, City of Montreal, on the "Importance of fluoridation as a public health measure". He said this had not yet been published but he thought that if the Secretary were to write to Montreal he could secure permission to reproduce the article in the proceedings of the Committee. It was AGREED that this should be done.

Note by Secretary - Dr. Bouillon's paper together with the conclusions of a paper by Dr. Ad. Groulx on the subject "Fluoridation and the Health Officer" appear in APPENDIX "T"

THE CHAIRMAN suggested that the Committee would give favourable consideration to an application for the continuance of project DR 13 if Dr. Smith found it possible to proceed with this work.

7. DR 22 - Dr. C.H. Best

"Part I - An Investigation of the Mechanism of Repair of the Supporting Structures of the Teeth; Part 2 - Fusospirochetal Infection and Pre-existing Inflammation"

The summary and the report on project DR 22 appear in APPENDIX "G"

THE CHAIRMAN invited Dr. Macdonald to present this report and he read the summary but said that he had not had an opportunity to study the report.

THE CHAIRMAN asked Dr. Ham and Dr. Macdonald to examine the report and to present their findings to the Committee at the next session. Next day they reported as follows:

DR. HAM said that the problem of reattachment was very important. Linghorne had shown that anchorage can be accomplished and he is now trying some very difficult experiments on dogs. Lately also he has turned to work on hormones probably because he has access to good material for research in this field. The grantee should probably be given another year on this work but ought to be urged to put the problem on a more practical basis to find something useful to dentistry. He felt that Linghorne tended to veer off into theory and to forget the importance of practical results.

DR. MacDONALD said he had reviewed Linghorne's reports. Linghorne has studied periodontal pockets 9-33 days after surgery finding some detachment and in other cases reattachment up to 1 mm. Seven dogs were studied as a source of bone to find whether osteoblasts were essential to bone formation. He hoped in his experimental work to show that any bone formed would not have come from osteogenic cells. Dr. Macdonald doubted the value of the technique used in this case.

Linghorne's third experiment had to do with the artificial production of periosteum. The last part of the report dealt with three groups of rats.

Dr. Macdonald agreed that the problem is important and probably should be completed. As far as the growth studies were concerned it was probably a good thing. He thought this would be a good field for Linghorne to continue to work in at the Banting Institute because they have a long-term program of growth-hormone studies in progress.

THE CHAIRMAN thanked Dr. Ham and Dr. Macdonald for their careful review of this report.

DR. GARVIN doubted that experiments in animals such as Linghorne was carrying on were likely to produce results that would be of any value in the study of similar processes in man. He thought the experimental work should be done on humans.

DR. BURTON referred to the sentences on page "G-8" (reading "Histologically .....accurately") and wondered why these were introduced.

DR. MacDONALD said he thought it was for the purpose of justifying the introduction of the growth-hormone studies into this project.

THE CHAIRMAN said that this project may have reached the stage where Linghorne should be regarded as a senior researcher in this field and that the Committee could not continue to support him as such indefinitely. He thought Dr. Best should be invited to attend the next meeting of the Committee.

DR. CHARRON said that the review by Dr. Ham and Dr. Macdonald of the report DR 22 was excellent and indicated the kind of preparation that should be made for the presentation of each report at a meeting of the Committee when the grantee himself is unable to attend.

DR. BURTON did not think the tables in report DR 22 contributed any information of value regarding the project.

8. DR 23 - Dr. C.P. Leblond

"Entry of Phosphate, Carbonate and Calcium Ions into Teeth, as shown with the Help of Radioactive Elements"

The summary and two appendices which constitute the report under project DR 23 appear in APPENDIX "B"

DR. HAM said he considered this work by Dr. Leblond was a magnificent contribution to dental research.

DR. CHARRON added that it was a subject which had been of great interest to many people for many years.

DR. MacDONALD referring to the third last line of para. 2 of the summary noted that there were "good reasons, etc." and said he would be interested in knowing what these reasons were.

9. DR 28 - Dr. O.J. Walker

"Investigation of Methods for Destroying the Organic Matter in Teeth, Bones, and other Materials prior to Determination of Fluorine Content"

The summary and report under DR 28 which were presented by Dr. H.R. MacLean appear in full in APPENDIX "M"

THE CHAIRMAN said that the wording of the title had been slightly changed by the grantee in order to secure better definition of the work in hand.

There was considerable discussion on this report regarding the tables shown on "M-2" and "M-3". The question was raised as to whether figures of recovery shown in the blank should be subtracted from the other figures given in the table.

THE SECRETARY was directed to bring these observations to the attention of the grantee and to ask whether any changes should be made in the report before it was included in the proceedings.

Note by Secretary - In a letter, dated 18 March 1953, from Dr. O.J. Walker, he writes: "In the tables on pages M-2 and M-3 the milligrams recovered is the actual amount found in the analysis."

10. DR 35 - Dr. J.B. Macdonald

"Experimental Fusospirochetal Infection with Mixtures of Pure Cultures"

DR. MacDONALD presented his summary and report on this project both of which appear in APPENDIX "D"

He said that the report was entirely unexciting and entirely negative. He felt that he had exhausted the possibilities of this investigation.

THE CHAIRMAN referring to the discussion in Min. 11, 16th Mtg., regarding Dr. Macdonald's request for support of the Committee in the publication of a monograph, asked whether anything further had been done in the matter. Dr. Macdonald said that he thought he might submit his manuscript either to one of the Canadian Journals of Research or to Rockefeller Monographs.

DR. BURTON suggested that the Canadian Society of Microbiologists

DR. CHARRON added that it was a subject which had been of great interest to many people for many years.

DR. MacDONALD referring to the third last line of para. 2 of the summary noted that there were "good reasons, etc." and said he would be interested in knowing what these reasons were.

9. DR 28 - Dr. G.G. Walker

"Investigation of Methods for Destroying the Organic Matter in Teeth, Bones, and other Materials prior to Determination of Fluorine Content"

The summary and report under DR 28 which were presented by Dr. H.R. Maclean appear in full in APPENDIX "M"

THE CHAIRMAN said that the wording of the title had been slightly changed by the grantee in order to secure better definition of the work in hand.

There was considerable discussion on this report regarding the tables shown on "M-2" and "M-3". The question was raised as to whether figures of recovery shown in the blank should be subtracted from the other figures given in the table.

THE SECRETARY was directed to bring these observations to the attention of the grantee and to ask whether any changes should be made in the report before it was included in the proceedings.

Note by Secretary - In a letter, dated 18 March 1953, from Dr. G.G. Walker, he writes: "In the tables on pages M-2 and M-3 the milligrams recovered is the actual amount found in the analysis."

10. DR 35 - Dr. A.B. Macdonald

"Experimental Phosphirochetal Infection with Mixtures of Pure Cultures"

DR. MacDONALD presented his summary and report on this project both of which appear in APPENDIX "D"

He said that the report was entirely unexciting and entirely negative. He felt that he had exhausted the possibilities of this investigation.

THE CHAIRMAN referring to the discussion in Min. 11, 16th Mtg., regarding Dr. Macdonald's request for support of the Committee in the publication of a monograph, asked whether anything further had been done in the matter. Dr. Macdonald said that he thought he might submit his manuscript either to one of the Canadian Journals of Research or to Rockefeller Monographs.

DR. BURTON suggested that the Canadian Society of Microbiologists

might be interested in doing something about it. Dr. N.E.Gibbons of the Division of Applied Biology, NRC, is the secretary of the Society.

11. DR 36 - Dr. G. Nikiforuk

"Demineralization of Hard Tissues by Organic Chelating Agents at Neutral pH's"

THE CHAIRMAN said that Dr. H.A. Hunter was present and would read the report on DR 36. The summary and report appear in APPENDIX "O"

DR. HUNTER said that while excellent results were obtainable by the use of nitric acid the work now reported suggested that "Versene" is even better as a chelating agent.

DR. HAM inquired whether it worked well on larger bone sections.

DR. HUNTER replied that it did. Versene takes longer to act than does nitric acid. He himself likes formula 2 as shown on page "O-3" (formic acid and sodium citrate).

THE CHAIRMAN observed that in the discussion on this project, (Min. 12, 16th Mtg.) Dr. Hamilton had asked whether Dr. Nikiforuk would be willing to undertake a broader project involving a study of all methods that are being suggested for demineralization of bone specimens. He asked if the present work covered the ground that Dr. Hamilton had in mind.

DR. HAMILTON said that this did not quite meet his needs. Like Dr. Ham he would be interested in seeing results of work done on larger pieces of bone.

12. DR 37 - Dr. R.E. Moyers

"Further Studies in the Electromyography of the Head, Face and Neck"

THE CHAIRMAN presented the summary of project DR 37 which appears in APPENDIX "P"

DR. BURTON thought this project represented good fundamental physiological work and that the grantee should be encouraged to continue his physiological studies as outlined in B(1) of the summary. He thought it was desirable to avoid extending this investigation under the Committee's auspices into the clinical field.

THE CHAIRMAN said the Secretary would bring this suggestion to Dr. Moyers' attention.

13. DR 38 - Dr. K.J. Paynter

"The Effect of Thiouracil on the Bone of the Jaws of Rats"

The summary and report on this project appear in APPENDIX "C"

These were presented by Dr. H.A. Hunter.

DR. HAM suggested that it would be useful for Dr. Paynter to discuss this project with Dr. C.P. Leblond. He felt also that it would be helpful to have the application reviewed by those who specialize in the field of endocrine research.

DR. HAMILTON said he was particularly interested in the effect of thyroid hormone on intercellular substances. He would like to know if in Paynter's experiments normal development of bone formation occurred. He thought there was something still to be learned on the effect of thyroid hormones. He would suggest that the attempt to stain pituitaries (last para. page "C-1") might be omitted as this would be a very long-term study.

DR. MacDONALD said that Dr. Paynter had a paper prepared for publication which contains a good review of the literature. He thought the second last paragraph on page "C-1" (As was stated ... normal) of the report might be dropped.

THE CHAIRMAN said that in the light of the interest shown in this report he would recommend that Dr. Paynter be invited to attend the next meeting of the Committee.

14. DR 39 - Dr. A.G. Racey

"A Histopathological Study of the Effects of a Potassium Deficiency and of a Pyridoxin Deficiency on the Teeth and Surrounding Structures of Dogs"

The summary of report DR 39 which was read by the Chairman appears in APPENDIX "F"

DR. HUNTER inquired how many dogs had been used in the experiment.

DR. HAMILTON wanted to know what practical application could be made of this research to a study of human beings since it is virtually impossible to have pyridoxin deficiency in man. (He was referring here to the concluding paragraph of the report).

Note by Secretary - Dr. E.W.McHenry says in his "Notes" that pyridoxin, originally known as B_6 has been found to be essential for protein metabolism in rats, dogs and swine, but not as yet for man.

DR. HUNTER said that a vast array of pathological pictures can be found within a normal range. The fact that the grantee had found no predentine was interesting.

THE CHAIRMAN said that there was no application for renewal but that he did not know whether this was a final report on the project or whether there would be a further contribution. He felt that much of the detail asked for by those who had raised questions regarding the report might be found in the two theses mentioned in the report.

15. DR 42 - Dr. G. Nikiforuk

"Studies in the Chemistry of the Saliva. II. The Reducing Property of Saliva towards some Oxidation-reduction Indicators"

The summary and report on this project which were presented by the Chairman appear in APPENDIX "L"

Some general discussion on this report took place.

16. DR 43 - Drs. S.G. Davis and H.R. MacLean

"Electropotentials between Dental Alloys"

Dr. H.R. MacLean presented a short summary which appears in APPENDIX "Q"

DR. MacLEAN said that so many variables occur in saliva that preliminary work is being done on saline solutions to determine the factors involved. The necessary equipment for the investigation has been purchased out of this year's grant.

DR. CHARRON inquired as to whether this was a clinical study but was assured that it has physiological significance and is basically a research relating to dentistry.

DR. HUNTER questioned the use of the word "insulating" as applied to cements.

17. DR 44 - Dr. A.H. Craven

"The Growth in Width of the Face and Head of the Macaque Monkey as Revealed by Vital Staining"

The summary of this report which was read by the Chairman appears in APPENDIX "I"

THE CHAIRMAN said that he thought it would be useful to invite Dr. Craven to be present at the next meeting of the Committee.

DR. HAM doubted whether the method using alizarin would be successful. The question is whether new bone is laid down in layers or through suture planes. He said workers in Chicago have used metal staples and other indicators in similar work since they believe there is intersutural growth. It is well known that alizarin stains new bone.

18. DR 45 - Dr. R.E. Moyers

"Study of the Incidence of Malocclusion"

A report on this subject appears in APPENDIX "J"

THE CHAIRMAN drew attention to Min. 22, 16th Mtg., in which it was agreed to make a preliminary grant to Dr. Moyers for the initiation of this investigation, but it was suggested to him then that he should make an effort to arrange for the provision of further support for this investigation after the end of the present fiscal year through the funds provided under the Public Health Grant. The Committee has now initiated the project and hopes that it will be carried on in accordance with the recommendation made at the 16th Meeting.

DR. BROWN said he thought it was being dealt with under the Crippled Children's Grant of the Public Health Appropriation.

19. DR 46 - Dr. R.E. Moyers

"An Analysis of Treated Orthodontic Cases"

A summary and report appear in APPENDIX "K"

THE CHAIRMAN said that work under this project was expected to be completed within six months.

20. DR 47 - Dr. H.A. Hunter

"The Pathological Characteristics of Experimental Fusospirochetal Infections"

A summary and report of this project appear in APPENDIX "N"

DR. HUNTER presented his own report and discussed it at some length. He asked for advice regarding the discrete pin-point spots which he had observed.

DR. HAMILTON said that they were probably evidence of coccidiosis of rabbits which is endemic in Toronto. He said also that interstitial nephritis occurs in these animals too, and that at Toronto they had given up trying to obtain rabbits locally for these reasons. He imported all his rabbits from Philadelphia, Pa., where they were free from these diseases.

DR. MacDONALD asked whether Dr. Hamilton would be willing to make diagnoses of the lesions found in this study. Dr. Hamilton said he would be glad to do so.

DR. THOMSON questioned the data in Table 1 on page "N-2" and comments on page "N-3".

DR. HUNTER asked permission to re-examine these two pages of his report and if necessary to supply the Secretary with a revised copy (which he subsequently did). AGREED.

21. Discussion on Reports

THE CHAIRMAN inquired whether the members had any comments to make on the overall picture of reports. He himself thought it had been very useful as a matter of policy to have grantees present from time to time to report on their work. He drew the attention of the members to information which had been compiled by the Secretary and which appeared in APPENDIX "R", 16th Mtg., showing a list of the grantees and subjects of their investigations from DR 1 to date. This list shows the active projects and those that have been completed. Tables in the same appendix show a summary of grants by years from 1945-46 to 1952-53 listing the grant number, the name of the grantee, and the amount of money awarded to him in each year. This was a very convenient general source of reference.

DR. RAE said that it would be helpful if the name of the grantee's department and the institution in which he works could be given in addition to his name on the reports from grantees. It often happens that members of the Committee are not able to identify grantees immediately. He thought also that some indication should be given as to when the contribution is a final report. Even when there is no further application being made by the grantee it is not always certain that the final report on the project has been submitted.

It was AGREED that this should be done, and the Secretary was instructed accordingly.

BRIG. WANSBROUGH felt that a personal report by the grantee should be made to the Committee at least once a year when the investigation in hand is regarded as one of the larger Committee projects.

DR. THOMSON said it would be very nice to have members of the Committee inform themselves on a particular project in advance of the meeting so that they might correspond with the grantee if necessary in order to clear up any matters on which they were in doubt after reading the report. The suggestion that had been made earlier in the meeting of having one or two members review a report in advance in order that they might present it to the Committee when the grantee was not present, seemed to him a very desirable procedure for increasing the efficiency of the Committee's operations.

DR. MacDONALD asked whether Dr. Hamilton would be willing to make diagnoses of the lesions found in this study. Dr. Hamilton said he would be glad to do so.

DR. THOMSON questioned the data in Table I on page "N-2" and comments on page "N-3".

DR. HUNTER asked permission to re-examine these two pages of his report and if necessary to supply the Secretary with a revised copy (which be subsequently did). AGREED.

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22. Finances (Min. 21, 16th Mtg.)

(i) Financial Standing of the Committee, 31 January 1953.

THE CHAIRMAN asked for a statement of Dental Committee's finances and the Secretary supplied the information given to him by the Treasury Division, 12 February, 1953. This statement appears on the following page (12a).

(ii) Estimates, 1953-54 (Min. 21(ii), 16th Mtg.)

THE CHAIRMAN reminded the Committee that the estimates for 1953-54 as recorded at the 16th Meeting are as follows:-

Associate Committee on Dental Research	
Grants in aid of research	$32,500
Fellowships	5,000
Committee Travel	2,500
Printing of Proceedings, etc.	200
Contingencies	500
	$40,700

(iii) Estimates, 1954-55 (Min. 21, 16th Mtg.)

THE CHAIRMAN asked for the members' views as to the estimates which would be required for the Committee's activities in 1954-55 in order that the Secretary might have the required information on hand when he is asked to submit the Committee's estimates in the autumn of 1953.

After some discussion it was MOVED by Dr. Thomson and SECONDED by Dr. Hamilton

That the Associate Committee on Dental Research respectfully submits to the National Research Council a request that financial provision be made for the Committee's activities in 1954-55 as follows:

Associate Committee on Dental Research	
Grants in aid of research	$32,500
Fellowships	5,000
Committee Travel	2,500
Printing of Proceedings, etc.	200
Contingencies	500
	$40,700 CARRIED.

DENTAL COMMITTEE FINANCES, JANUARY 31, 1953

1.	Total grant, 1952-53		$40,700.00
2.	Carried over from 1951-52 for purchase of goods ordered but not delivered on 1 April 1952		1,047.61
3.	Grants awarded 1952-53		25,410.00
4.	Total available to grantees		$26,457.61
5.	Less funds in the possession of grantees, 1 April 1952		2,597.14
6.	Net		23,860.47
7.	Advanced to grantees to 31 January 1953		20,867.59
8.	Balance payable to grantees		2,992.88
9.	Other expenditures and commitments (Travel $1,388.77 contingencies 199.38)	$1,588.15	
	Balance due on Dr. Liu's Fellowship award	220.00	1,808.15
10.	Unexpended balance available for disposition by the Committee		$13,481.85

Information supplied by the
Treasury Division,
Trust Fund Section,
National Research Council.

12 February, 1953.

DENTAL COMMITTEE FINANCES, JANUARY 31, 1953

1.	Total grant, 1952-53	$40,700.00
2.	Carried over from 1951-52 for purchase of goods ordered but not delivered on 1 April 1952	1,047.61
3.	Grants awarded 1952-53	25,410.00
4.	Total available to grantees	$26,457.61
5.	Less funds in the possession of grantees, 1 April 1952	2,597.14
6.	Net	23,860.47
7.	Advanced to grantees to 31 January 1953	20,867.59
8.	Balance payable to grantees	2,992.88
9.	Other expenditures and commitments (Travel $1,388.77 contingencies 199.38)	$1,588.15
	Balance due on Dr. Liu's Fellowship award 220.00	1,808.15
10.	Unexpended balance available for disposition by the Committee	$13,481.85

Information supplied by the
Treasury Division,
Trust Fund Section,
National Research Council.

12 February, 1953.

23. Fellowships (Min. 20, 16th Mtg.)

THE CHAIRMAN said that the fellowship plan inaugurated by the Committee had not worked out very satisfactorily as yet. Two fellowships had been awarded and one of them held by Dr. Shklar had undoubtedly yielded good results. The second fellowship held by Dr. Willa Liu Chou had not been completed owing to circumstances beyond the control of the Committee and the fellowship holder. No applications for fellowships have since been received.

DR. BURTON thought the Committee should seriously consider this lack of applications for fellowships, and suggested that it might be because the amounts offered were too low. He did not think a graduate dentist could afford to take $1500 as compensation for a year's work in research. Perhaps it would be better to be more selective and to make higher offers to fewer persons. He thought the most important thing for the Committee to bear in mind was that no brilliant candidate should be denied the opportunity for graduate research because of lack of funds.

DR. RAE wanted to know where a graduate dentist who had completed one year of graduate research under a fellowship was likely to find a job other than in general practice. He thought the lack of opportunity for continuity in research was one of the deterrent factors in keeping graduate dentists from applying for the Committee's fellowships.

DR. THOMSON said that a year's graduate work in research was likely to be more of an asset to a medical graduate than to a graduate in dentistry.

DR. MacDONALD said that a graduate could not live on the amount provided under a fellowship and he agreed that there was nothing for a fellowship holder to look forward to in Canada apart from teaching and the number of vacancies occurring on the staff of the dental schools was relatively small. He said the Canadian Dental Association offered grants to enable dental graduates to take further training preparatory to coming back to a Canadian dental school in a staff position. He felt that the lack of applications for Committee fellowships arose as much from the lack of jobs to be had following the graduate work as from the limited appropriation offered to fellows.

DR. BURTON raised the question as to whether a graduate assistant (as for example Dr. Linghorne in DR 22) might not be given a fellowship instead of being employed on a half-time basis under a grant.

THE CHAIRMAN said that it was apparent the Committee had a very difficult problem before it and he said that President Steacie had very kindly offered to meet with the Committee for the discussion of any special problem which might arise. He suggested therefore that Dr. Steacie be invited to join in this discussion.

23. Fellowships (Min. 20, 16th Mtg.)

THE CHAIRMAN said that the fellowship plan inaugurated by the Committee had not worked out very satisfactorily as yet. Two fellowships had been awarded and one of them held by Dr. Shklar had undoubtedly yielded good results. The second fellowship held by Dr. Willa Lin Chou had not been completed owing to circumstances beyond the control of the Committee and the fellowship holder. No applications for fellowships have since been received.

DR. BURTON thought the Committee should seriously consider this lack of applications for fellowships, and suggested that it might be because the amounts offered were too low. He did not think a graduate dentist could afford to take $1500 as compensation for a year's work in research. Perhaps it would be better to be more selective and to make higher offers to fewer persons. He thought the most important thing for the Committee to bear in mind was that no brilliant candidate should be denied the opportunity for graduate research because of lack of funds.

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THE CHAIRMAN said that it was apparent the Committee had a very difficult problem before it and he said that President Steacie had very kindly offered to meet with the Committee for the discussion of any special problem which might arise. He suggested therefore that Dr. Steacie be invited to join in this discussion.

Dr. Steacie, who very kindly accepted the Chairman's invitation to join the meeting and take part in this discussion, was told by the Chairman that the Committee was gravely concerned about the lack of applications for fellowships. Several examples of possible candidates for fellowships were mentioned but no applications had been received possibly because of the low salary offered and partly because of the lack of opportunity for appointments following graduate research.

DR. STEACIE said the National Research Council has a fair budget for research, but is definitely opposed to giving grants to university professors or teachers for their own support. A member of a university staff should not be paid a salary out of a committee grant. The scholarship funds provided by the Council permit a few hours of teaching to be done but nothing approaching a half-time basis. If a larger part of the time of university employees were paid for under grants from the Council it might easily develop into a scheme for increasing the salaries of university staff members; and although he felt that university salaries were in general too low, he was quite sure the National Research Council should not be used as the instrument to finance a general rise in university salary levels.

THE CHAIRMAN pointed out that the Division of Medical Research offers fellowships of a value of $1500 to medical graduates and from $1800 to $3000 to medical graduates who have had experience in research. The corresponding dental fellowships offer $1500 to graduates and $1600 to $2500 to graduates who have had experience. The dental fellowships had been based in the first instance on the awards made for medical scholarships but the ratings on medical fellowships have since been increased and he thought perhaps the Dental Committee should put forward a similar recommendation. He noted also that the Division of Medical Research offered a limited number of senior medical research fellowships of an annual value from $3000 to $5000 which were renewable annually for a period not exceeding five years. Applications for this class of fellowship must be made by the president of the university who must undertake to provide research facilities and an honorary appointment within the structure of the medical school.

PRESIDENT STEACIE thought it should be possible for the Dental Committee to follow the Medical Division practice in respect of fellowships. He thought there was a closer relationship between dental and medical graduates than with graduates in the basic sciences. He went on to say that in engineering it is not the practice to put much stress on higher degrees and graduate research experience. In other words the engineer is considered to be fully trained and ready to go to work when he leaves the university. This is also true in dentistry. In medicine it is recognized that a graduate must do at least one year's interneship, and frequently more than one year.

In chemistry and physics it is generally accepted that a man is not fully trained in his science until he has reached the Ph.D. level. In the matter of fellowships the Council's policy is to provide for the training of the candidate up to the Ph.D. level. At that point, however, the philosophy changes and becomes more selective looking to the provision of good support for a limited number of individuals, who have demonstrated their research ability.

THE CHAIRMAN thanked President Steacie for his helpful advice.

24. Fellowship Plans, 1954-55

THE CHAIRMAN said that in the light of the discussion with the President it appeared to be in order to recommend revision of the regulations governing graduate dental research fellowships by increasing the amount of the annual awards.

DR. THOMSON suggested that the Committee ask Council for authority - (i) to raise the value of the two ordinary fellowship classes and (ii) to establish provision for one postdoctorate fellowship equivalent to the senior medical fellowship.

After some discussion it was MOVED by Dr. Bagnall and SECONDED by Dr. Burton,

> That the Associate Committee on Dental Research, having been unsuccessful in two years in obtaining applications for the fellowships which it has been offering to graduates in dentistry, is of the opinion that the annual awards, provided under these fellowships of $1500 for graduates and $1600 to $2500 for graduates who have had experience in research, are too low to attract graduates into this field of research;
>
> The Committee therefore recommends to Council that authority be granted to revise item 12 of the regulations on this subject to read as follows:-
>
> 12. Fellowships of the value of $1800 per annum will be open to graduates in dentistry with high academic standing.
>
> Fellowships of the value of $2000 to $3000 per annum will be open to graduates in dentistry who have had experience in research work. The value of the Fellowship will depend on the candidate's training and achievement in an original research.

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Fellowships of the value of $2000 to $3000 per annum will be open to graduates in dentistry who have had experience in research work. The value of the Fellowship will depend on the candidate's training and achievement in an original research.

The Committee also requests that authority be granted for the award of one senior dental research fellowship of an annual value of $3000 to $5000 renewable annually for a period not exceeding five years and suggests that the terms under which this senior dental research fellowship is awarded should be similar to those provided on NRC form 106A-1(21.8.51) covering the award of senior medical research fellowships.

CARRIED.

THE CHAIRMAN pointed out that provision had been made (Min. 20, 16th Mtg.) for Dr. Moyers to arrange for some one to be sent to Cleveland and Iowa City to secure the information needed by Dr.Liu in connection with the completion of her fellowship study but that unfortunately Dr. Moyers had been unable to find anyone to undertake this task. It was AGREED that if no action were taken prior to 31 March, 1953, the fellowship should be closed.

25. Meeting of the Executive

THE CHAIRMAN said a meeting of the Executive would be held at 8 p.m., Monday, 2 March, 1953, in Room 427, Chateau Laurier, to consider applications for grants, nominations for the membership of the Committee, and date of next meeting.

The meeting adjourned at 5:30 p.m., on the first day and reconvened at 9 a.m., on Tuesday, 3 March, 1953.

SECOND SESSION

26. Attendance

Dr. R.G. Ellis, Chairman
Dr. E.W.R. Steacie, President NRC
Dr. J.S. Bagnall
Dr. H.K. Brown
Dr. A.C. Burton
Dr. E. Charron
Dr. M.H. Garvin
Dr. A.W. Ham
Dr. J.D. Hamilton
Dr. L.A. Kilburn
Dr. J.B. Macdonald
Dr. R.H. McDougall
Dr. H.R. MacLean
Dr. E. Pagé
Dr. J.J. Rae
Dr. D.L. Thomson
Brig. E.M. Wansbrough

Mr. S.J. Cook, Secretary

By Invitation

Dr. H.A. Hunter

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By Invitation

Dr. H.A. Bunter

27. Overhead on Research Grants

THE SECRETARY reported that he had received a letter from Dr. J.B. Macdonald, Chairman of the Division of Dental Research, Faculty of Dentistry, Toronto, Ont., suggesting that the question of overhead charges on research grants be placed on the agenda for this meeting. He said he had welcomed this suggestion because it provided an opportunity for discussion of a subject that was very much in the minds of all university people who were concerned with the rising costs of administration generally and who had a specific interest in the matter as it concerns grants-in-aid from the National Research Council.

He said that this was a subject which had been considered by the National Research Council at its meeting 10 November, 1952. Dr. G.P. Gilmour, President, National Conference of Canadian Universities, had written to President Steacie, transmitting a resolution which had been passed by the Universities' Conference in June 1952, asking that organizations such as the National Research Council consider the possibility of compensating the universities to which they make grants-in-aid of research for the overhead expenditures incurred in relation to the conduct of such research.

The Council, while fully appreciating the difficulties of the situation, had reached the unanimous view that "the policy followed in the past on this question is reasonable and fair to the universities and that it should be continued."

THE CHAIRMAN said that the information given by the Secretary seemed to preclude any action being taken by the Committee in this connection. Everyone realized that the universities were put to some additional expense in dealing with the extra funds received for research from the National Research Council and it was also known that certain other organizations have adopted the policy of increasing their grants to universities by a certain percentage as an offset to the rising costs of administration.

Some discussion ensued but it was finally AGREED to defer action in the hope that the Universities' Conference might renew its application to the National Research Council.

28. Applications for Grants, 1953-54 (Min.31, 15th Mtg.)
(Min.22, 16th Mtg.)

THE CHAIRMAN said that the Executive had reviewed all applications for grants including renewals and new applications and had prepared a list of recommendations regarding them for the consideration of the Committee. He pointed out that each member of the Committee should feel quite free to express his views whether they were in agreement with the recommendations put forward by the Executive or not. Each application was then considered by the Committee and action was taken as recorded on following page.

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(i) RENEWALS

Grant No.	Grantee	Amount $
DR 1	Dr. J. J. Rae	1680
	Dr. Rae's original application was for $1580 but the Committee agreed to allow him an extra $100 for his technician.	
DR 22	Dr. C.H. Best	4200
	The Committee was of the opinion that it is not desirable to make substantial contributions to the income of relatively senior scientific workers for more than a limited number of years for the obvious reason that such workers may come to depend considerably on this income whereas the granting body is by its terms of reference required to reconsider its commitments annually and has no certain continuance. A letter in this sense is to be sent to the grantee with a view of terminating Dr.Linghorne's appointment at the end of the coming year.	
DR 23	Dr. C.P. Leblond	3000
DR 28	Dr. O.J. Walker	1000
DR 35	Dr. J.B. Macdonald	3610
	The question was raised again as to whether it would not be economical to buy a deep-freeze instead of purchasing carbon dioxide ice each year, but the Chairman said he thought this would be the final year in which the CO_2 ice would be needed.	
DR 37	Dr. R.E. Moyers	300

(1) RENEWALS

Grant No.	Grantee	Amount $
DR 1	Dr. J. J. Rae	1650
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DR 29	Dr. C.P. Leblond	3000
DR 28	Dr. O.J. Walker	1000
DR 35	Dr. J.B. Macdonald	3610
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DR 37	Dr. R.E. Moyers	300

Grant No.	Grantee	Amount $
DR 38	Dr. K.J. Paynter	1000
	It was hoped this project could be wound up during the year.	
DR 43	Dr. H.R. MacLean and Dr. S.G. Davis	1025
	It was suggested that this study should be cleaned up as a pilot investigation this year.	
DR 44	Dr. A.H. Craven	1350
DR 46	Dr. R.E. Moyers	520
	To be completed this year.	
DR 47	Dr. H.A. Hunter	2200
	Total renewals	$19,885

(ii) NEW APPLICATIONS

Grant No.	Grantee	Short Title	Amount $
DR 48	Dr. G. Nikiforuk Associate Professor of Pedodontics, University of Toronto	The effect of topical application of silicones in protecting teeth against acid solutions in vitro and dental caries produced in vitro.	2240
	The original application was for $2740 but the Executive recommended that the second phase of the investigation dealing with fluoridation of milk and its effect on the caries incidence of the cotton rat be dropped.		

DR. BROWN and DR. MacDONALD both thought it was important to work on the second problem and that it should be possible to obtain good results in eighteen months. Nutritionists are very much interested in the question of fluoridation of milk.

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DR. GARVIN said he knew of one paper in which the claim is made that fluoridation of milk is not practical because of the cost.

DR. RAE thought there was so much calcium in milk that any sodium fluoride added to the milk would be co-precipitated with the calcium and that the necessary fluorine analyses would be long and tedious.

After some further discussion it was AGREED to accept the recommendation of the Executive that the study be limited this year to the first project and that Dr. Nikiforuk be advised that if he is so inclined he might file a new application to carry out the fluoridation experiments. This action was APPROVED on a motion MOVED by Dr. Thomson and SECONDED by Dr. Bagnall.

Grant No.	Grantee	Short Title	Amount $
DR 49	Dr. A.H. Craven Lecturer, Faculty of Dentistry, McGill University, Montreal. After the explanatory matter accompanying the application had been read it was AGREED to ask the grantee for a more specific and descriptive title.	Posture of the head in man	400
DR 50	Dr. J.B. Macdonald Chairman, Division of Dental Research, Faculty of Dentistry, Toronto, Ont.	The cultivation and characteristics of bacteroides nigrescens	400
DR 51	Dr. R.L.deC.H.Saunders, Prof. of Anatomy, Dalhousie University, Halifax, N.S.	Study of human dental pulp blood vessels by X-ray micro-arteriographic methods	1200

(iii) APPLICATIONS NOT GRANTED

(a)	Dr. C.B. Weld, Prof. and Head of the Department of Physiology, Dalhousie University, Halifax, N.S.	The use of vital stains for identification of the macrophages in the pulp-tissue of the rat incisor.	(4760)

DR. HAM thought that some of this work might have been done before.

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Grant No.	Grantee	Short Title	Amount
			$
DR 49	Dr. A.H. Craven Lecturer, Faculty of Dentistry, McGill University, Montreal.	Posture of the head in man	400
DR 50	Dr. W.B. Macdonald Chairman, Division of Dental Research, Faculty of Dentistry, Toronto, Ont.	The cultivation and characteristics of bacteroides <u>pigrescens</u>	400
DR 51	Dr. R.L.deC.H.Saunders, Prof. of Anatomy, Dalhousie University, Halifax, N.S.	Study of human dental pulp blood vessels by X-ray micro-arteriographic methods	1200

(111) APPLICATIONS NOT GRANTED

(a)	Dr. C.B. Weld, Prof. and Head of the Department of Physiology, Dalhousie University, Halifax, N.S.	The use of vital stains for identification of the macrophages in the pulp-tissue of the rat incisor.	(4760)

DR. HAM thought that some of this work might have been done before.

DR. THOMSON noted that the research assistant is a senior scientist and thought the grantee should be advised that it is not the policy of the Committee to support senior scientific personnel under grants. He thought moreover that while the team was good it needed a larger ball; the project is not big enough to warrant the half-time employment of a man of Dr. A.G. Nutlay's attainments.

It was MOVED by Dr. Macdonald and SECONDED by Dr. Thomson

That the grantee be asked to re-submit his application expanding the project sufficiently to warrant the amount of the grant requested, and

That the Executive be authorized to deal with the new application. CARRIED.

(b)	Dr. M. Doreen Smith University of Toronto	An examination of dietary ingestion of fluorine in Newfoundland	(2600)

On discussion with Dr. Smith it was AGREED that she should withdraw this application. The Executive recommended that favourable consideration be given to an application for a renewal of Dr. Smith's grant under DR 13 for further work on teeth from the Brantford area. It was expected that Dr. Smith would subsequently submit another application which could be dealt with by the Executive.

(c)	Dr. E.E. Johns, Faculty of Dentistry, University of Toronto	A study of different ways in which the primary dentition is transferred to the permanent dentition	(600)

The candidate proposed to spend his entire grant in travel to various museums (New York, Chicago, Ann Arbor, Ottawa, and Boston). The Executive recommended that no award be made under this application but that the Chairman be authorized to make a travel advance to Dr. Johns for a visit to one museum or one group of museums and subsequently for visits to other museums if he considered that the results obtained in the first trial visit warranted continuance of the plan. AGREED.

29. Summary of Applications

	No.	Value
(i) Renewal applications approved	11	
(ii) Total value		$19,885
(iii) New applications approved	4	
(iv) Total value		4,240
(v) Total awards	15	$24,125

In addition to the foregoing totals there will probably be at least two other applications, one from Dr. Weld and one from Dr. Smith which may amount to another $5000.

It was MOVED by Dr. McDougall and SECONDED by Dr. Rae

That the list of applications as amended above be approved. CARRIED.

30. Special Travel Grants (i) International Association for Dental Research

THE CHAIRMAN reminded the members that special authorization had been given by the Committee a year ago to cover the award of travel grants for certain members of the Committee who wished to attend the International Association for Dental Research which was held in Colorado Springs.

It had been the intention of the Committee to provide similar authorization for the attendance of a limited number of members at the corresponding meeting in 1953 which is to be held in Philadelphia in March, but this matter was not dealt with at the September meeting of the Committee.

Consequently, a letter ballot of the Executive had been taken as a result of which approval had been given to the award of five dental research special travel grants each for an accountable amount not to exceed $150. Notification of this offer had been sent to all grantees and members of the Committee in October 1952.

Seven applications were received. The question arose as to whether a ballot should be taken or whether some other action was desirable. All of the applicants were grantees under the Committee and were exceptionally well qualified to represent the Committee at the International Meeting.

It was MOVED by Dr. Burton and SECONDED by Dr.Hamilton

That in view of the qualifications of the applicants for special travel awards, the number of grants provided by the Committee be increased from five to seven each for an accountable amount not to exceed $150 to be expended in connection with the attendance of grantees at the Annual Meeting of the International Association for Dental Research in Philadelphia, 20-22 March, 1953, and

That these awards be made to the following:- Dr. H.A. Hunter, Dr. C.P. Leblond, Dr. J.B. Macdonald, Dr. R.E. Moyers, Dr. G. Nikiforuk, Dr. K.J. Paynter, Dr. J.J. Rae CARRIED.

30. (ii) American Association of Anatomists

THE CHAIRMAN stated that an application had been received from Dr. R.C. Greulich, Department of Anatomy, McGill University, stating that he had submitted an abstract of the data contained in his report on DR 23 (with Dr. C.P. Leblond) to the Anatomical Record and that he would like to be able to present these results at the forthcoming meeting in Columbus, Ohio, of the American Association of Anatomists. He inquired whether the Committee would be willing to defray his expenses on this occasion which he estimated at about $110.

On consideration of this request it was MOVED by Dr. Hamilton and SECONDED by Dr. Ham

> That the application for a travel grant from Dr. R.C. Greulich to permit his attendance at the Columbus, Ohio, meeting of the American Association of Anatomists, March 1953, be approved on an accountable basis in an amount not to exceed $150. CARRIED.

The total amount of travel funds encumbered in these eight special grants was $1200.

31. Air Travel, 1953-54 (Min. 28, 15th Mtg.)

It was MOVED by Brig. Wansbrough and SECONDED by Dr. Bagnall

> That air travel to meetings of the Committee in 1953-54 be authorized for all members of the Committee, to be used at their discretion. CARRIED.

32. Membership of the Committee (Min. 30, 15th Mtg.)

THE CHAIRMAN said that five members having completed two terms each would be ineligible for reappointment for at least one year. There would be two vacancies for dentists and three for representatives of the basic sciences to maintain the Committee establishment on the authorized basis. He said he was always sorry to see any members come to the end of their term on this Committee because everyone devoted himself to the interests of the Committee and the group as a whole was an excellent working team. On his own behalf and in the name of the Committee he wished to express sincere appreciation of the services which had been rendered during their terms of office by the members who are retiring at the end of this year. The two dentists are: Dr. Garvin and Dr. MacLean and the representatives of the basic sciences are: Dr. Best, Dr. Rae and Dr. Thomson.

The Executive had given some consideration to this subject and had AGREED to put forward the following names for consideration by the Committee:

Dentists — Dr. C.D. Husband, Red Deer, Alberta, a practising dentist;

Dr. A.G. Racey, Associate Professor of Oral Pathology, McGill University;

Basic Sciences

Dr. J. Aldous, Head of the Department of Pharmacology, Dalhousie University;

Dr. J.M. Beveridge, Head of the Department of Biochemistry, Queen's University;

Dr. R.E. Haist, Professor of Physiology, University of Toronto;

Dr. M.M. Hoffman, Department of Medicine, McGill University.

He invited the Committee to make further nominations.

A general discussion ensued in the course of which several members suggested various names as follows:

Dr. H.G. Thode, Principal of Hamilton College, Hamilton, Ont.

Dr. C.P. Leblond, Professor of Anatomy, McGill University.

or alternatively, Dr. H. Copp, Professor of Physiology, University of British Columbia.

Dr. Howard Merkley, Winnipeg, Man.

DR. GARVIN suggested that consideration be given to nominating the editor of the Journal of the Canadian Dental Association in an ex-officio capacity.

THE CHAIRMAN said that this subject had been discussed by the Executive and it had been decided that it would be unwise to extend the list of ex-officio members beyond its present scope which includes only Service Department heads of dental services. There was no reason why the editor of the Journal of the Canadian Dental Association could not be considered on his own merits as a possible candidate for membership in the Associate Committee.

The Executive had given some consideration to this subject and had AGREED to put forward the following names for consideration by the Committee:

Dentists Dr. C.D. Husband, Red Deer, Alberta, a practising dentist;

Dr. A.G. Nacey, Associate Professor of Oral Pathology, McGill University;

Basic Sciences

Dr. J. Aldous, Head of the Department of Pharmacology, Dalhousie University;

Dr. J.M. Beveridge, Head of the Department of Biochemistry, Queen's University;

Dr. R.E. Hulet, Professor of Physiology, University of Toronto;

Dr. M.M. Hoffman, Department of Medicine, McGill University.

He invited the Committee to make further nominations.

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DR. HAM suggested that Dr. E.W. McHenry, Nutritionist, School of Graduate Studies, Toronto, Ont., would be an asset to the Committee.

THE CHAIRMAN said that in view of the number of candidates proposed he thought it would be desirable to take a ballot on the Committee's preferences and this was done with the following results:-

Nominated as dental candidates:	Dr. A.G. Racey Dr. C.D. Husband
Nominated as representatives of the basic sciences:	Dr. J. Aldous Dr. J.M. Beveridge Dr. C.P. Leblond (or Dr.H.Copp if Dr. Leblond is unable to act)

It was MOVED by Dr. MacLean and SECONDED by Dr.Hamilton

That the Associate Committee on Dental Research recommend to the National Research Council that the five vacancies arising from the retirement of members from the Committee at 31 March, 1953, be filled by the appointment of Dr. C.D. Husband and Dr. A.G. Racey, as representatives of the dental profession; and Dr. J. Aldous, Dr. J.M. Beveridge and Dr. C.P. Leblond (or if he is unable to act, Dr. H. Copp), as representatives of the basic sciences. CARRIED.

33. Appreciation of Services

It was MOVED by Brig. Wansbrough and SECONDED by Dr.Charron

That the Associate Committee on Dental Research place on record an expression of its sincere appreciation for the services rendered to the Committee and in the promotion of dental research in Canada by the five members who are retiring from the Committee at the end of this year, and that the Secretary be instructed to send a letter to each of them conveying the Committee's appreciation of their services and the continued good wishes of their colleagues. CARRIED.

34. Suggested Research Projects (Min. 24, 16th Mtg.)

THE CHAIRMAN drew attention to the listing of suggested research projects which is carried as a standard appendix to the proceedings of the Committee and asked if there were any items to be added.

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35. Date of Next Meeting (Min. 26, 16th Mtg.)

THE CHAIRMAN said that this subject had been considered by the Executive. It had been pointed out that the meetings of the Canadian Dental Association are to be held in Montreal in October, 1953, and it was decided to recommend that the next meeting of the Associate Committee on Dental Research be also held in Montreal, on Thursday, 22 October, 1953, immediately following the meetings of the Canadian Dental Association.

He said that Dr. Charron had very kindly offered to arrange for the accommodation of the Committee in the School of Dentistry, University of Montreal, on that occasion.

The offer of the University facilities made by Dr. E. Charron was very much appreciated by the members and it was AGREED to accept the recommendation of the Executive and to hold the next meeting of the Committee in the School of Dentistry, University of Montreal, Thursday, 22 October, 1953.

The Meeting adjourned at 1:15 p.m.

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APPENDIX "A"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

PAPERS PUBLISHED

Committee Paper No.	Title and Author	Reference
1	New aspects of periodontal research, by H.K. BOX	Presented at National Convention of Canadian Dentists, Toronto, 29 May, 1946. (App. "C", 5th Mtg. A.C.D.R., 25 Feb., 1947).
2	Concerning the pathogenesis of periodontal disease, by H.K. BOX	J. Periodontology, 19: 11-20. 1948. (App. "G", 7th Mtg. A.C.D.R., 2 Mar., 1948).
3	The effect of various inorganic salts on the solubility of calcium phosphate, tooth enamel and whole teeth in lactic acid, by J.J. RAE and C.T. CLEGG	J. Dent. Res. 27: 52-53. 1948.
4	Changes in the calcium and phosphate concentrations of saliva and inorganic salt solutions on shaking with calcium phosphate, by J.J. RAE and C.T. CLEGG	J. Dent. Res. 27: 54-57. 1948.
5	An improved method for processing acrylic dentures, by D.R. CHRISTIE	J. Can. Dent. Assoc., 14: 303-317. 1948. (App. "R", 8th Mtg., A.C.D.R., 26 Oct., 1948).
6	The therapeutic properties of periodontal cement packs, by W.J. LINGHORNE and D.C. O'CONNELL	J. Can. Dent. Assoc., 15: 199-205. 1949.
7	Phosphatase in saliva, by J.T. DENTAY and J.J. RAE	J. Dent. Res., Feb. 1949. 4pp.
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11	The corrosion of cobalt chromium alloys in commercial cleaning agents, by D.E.R. COGGLES, O.J. WALKER and H.R. MACLEAN	J. Can. Dent. Assoc., 16: 75-82, 1950.
12	The relation between buffering capacity, ciscosity and lacto-bacillus count of saliva, by J.J. RAE and C.T. CLEGG	J. Dent. Res. 28: 589-593. 1949.
13	Mineralization of the growing tooth as shown by Radio-phosphorus autographs (17692), by L.F. BELANGER and C.P. LEBLOND	Proc. Soc. Expt. Biol. Med. 73: 390-391. 1950.
14	Radio-autographic visualization of bone formation in the rat, by C.P. LEBLOND, G.W. WILKINSON, L.F. BELANGER and J. ROBICHON	Am. J. Anat. 86: 2. 1950.
15	Fluoride studies related to the human diet, by MARY P. HAM and M. DOREEN SMITH	Can. J. Research, F, 28: 227-233, 1950.
16	Studies in the regeneration and reattachment of supporting structures of the teeth. I. Soft tissue reattachment, by W.J. LINGHORNE and D.C. O'CONNELL	J. Dent. Res. 29: 419-428. 1950
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20	Acrylic fillings - General comments and some experimental data, by D.R. CHRISTIE	J. Can. Dent. Assoc., 17: 427-435. 1951.
21	Ammonia production in saliva, by R.M. BALLANTYNE, C.T. CLEGG, J.J. RAE, and F.H. LAWFORD	J. Dent. Res. 30: 385-387. 1951.
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23	Radioactive isotopes in dental research, by J.P. LUSSIER and A.D'IORIO	The Brit. Dent. Annual, 1952.
24	Determination of fluoride in water, by the aluminum-hemotoxylin method, by MARGARET J. PRICE and O.J. WALKER	Anal. Chem., 24: 1593. 1952.

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March, 1953.

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APPENDIX "B"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

February, 1953

Subject: Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radio-active elements.

Grantee: Dr. C.P. Leblond

Project No.: DR 23 Amount of Grant: $3000

SUMMARY OF WORK

The two main aspects of this work are the study of the mineral deposition of calcium-phosphate in the tooth (carried out mainly by radio-autographic studies of P^{32} and Ca^{45}) and the investigation of the formation of matrix (carried out with the help of C^{14}).

The formation of the mineral elements of teeth has given rise to an article published several years ago - Committee Paper No. 13, "Mineralization of the growing tooth as shown by radio-phosphorus autographs (17692)", by L.F. Bélanger and myself - reprinted from Proceedings of the Society for Experimental Biology and Medicine, 73: 390-391. 1950 - Since then, a number of interesting findings have been obtained and particularly, it was shown that the behaviour of calcium and phosphate was identical as far as could be judged from radio-autographic results. The minerals are deposited as a layer on the inner surface of the tooth and, therefore, appear as bands on sections subjected to radio-autography. No articles have been published as yet, due to a few surprising findings. Thus, the bands observed on radio-autographs often seem to become more widespread and less precise with time (compare figures 1 and 2 of report of Y. Kumamoto). This and similar findings point to the conclusion that crystals of dentine are not necessarily stable but may dissolve out and their content may precipitate again on neighbouring crystals. The interpretation of these results is most difficult. There are good reasons for thinking that mechanical factors, particularly pressure, are responsible for these unexpected findings. This possibility is being investigated.

The question arose as to whether or not the variations observed in the pattern of deposition of the mineral elements would also take place with the deposition of the matrix. The work of Mr. Greulich on the subject suggests that this is not the case. Thus, the enclosed figure of a C^{14} radio-autograph from an animal given two injections of radio-carbon at three-day intervals shows two well-defined lines corresponding to the matrix formed at each one of these times. In other words, it appears that the matrix formed has remained where it was initially deposited.

In conclusion, the trend of the results is that in the teeth of very young animals, matrix is stable but the mineral elements have a certain degree of lability (presumed due to the effect of mechanical factors).

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February 1953

by

Yurika Kumamoto

Project No: DR 23

In the previous report, September, 1952, results of radio-autographs of molars and incisors obtained with radio-calcium injected subcutaneously into newborn rats were presented. Since then, further work has been done along the same line and, in addition, was extended to older animals and a series with radio-phosphorus was carried out.

MATERIALS AND METHODS

To compare the fate of radio-calcium in older animals, a group of three-day old rats was sacrificed at 3, 6, 9, and 12 days after injection of this element. In addition to providing an older group for observation, these also had the advantage of having teeth which were larger than those of the newborn. To check the rate of the two radio-isotopes, P^{32} and Ca^{45} under identical conditions, two groups of rats were set up, one group of which received P^{32} subcutaneously (25 µc per rat), and the other which received Ca^{45} (Approx. 4 µc), also subcutaneously. These two groups were sacrificed at intervals of 3, 6, 9, 12, and 15 days after injection. All the jaws with teeth were removed under ether anesthesia and immediately fixed in a mixture of 95% alcohol:formaldehyde::3:1 with a pH between 7 and 8. Unstained and alizarin stained longitudinal sections of the lower incisors and upper molars were made and radio-autographed by the coating method (Gross et al, 1951). Jaws of younger rats were embedded either in paraffin or celloidin and those of older rats in methyl methacrylate. In the latter method, a carborundum wheel was used to cut the blocks; these were then filed, washed and glued to microscope slides and radio-autographed in the usual way.

OBSERVATIONS AND RESULTS

General observations applicable to all the results are firstly, the presence of two gradients of radio-autographic reaction, one the disto-proximal or basal-coronal gradient and the other, radially, which in the dentine and enamel is through the thickness. The radio-autographic intensity invariably increases from the proximal or basal region to the distal or coronal region. Radially, in the dentine, the reaction increases from the pulpal margin to the dentino-enamel junction, and in the enamel, the reaction decreases from the dentino-enamel junction to the surface of the enamel.

In the molars, there is an antero-posterior gradient, which accounts for the fact that the second molar is quite behind in the development of all of its structures. In the experiment, therefore, only the first molars were examined.

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All the observations showed an autographic reaction which was greater in the dentine than in the enamel. Predentine of molars and incisors showed no significant reaction.

Radio-autographs of three-day old rats injected with radio-calcium

The dentine and enamel of molars showed two gradients of autographic reaction, the basal-coronal and radial. The dentine also showed a band of autographic reaction just before the dentino-enamel junction, this being most clear about the tips of the molar cusps where the matrix is thicker. The band was observed up to 6 days after injection; 9 days after injection it was no longer present. The intensity of the autographic reaction was lighter in the enamel than in the dentine and showed no band.

The dentine of the tip of the incisors showed a band of reaction in an area about midway between the odontoblasts and the dentino-enamel junction. Basally, there was only the radial and proximo-distal gradient to be observed. This band, also present up to 6 days after injection, was no longer present 9 days after injection of Ca^{45}. At this time, the dentine showed a reaction which increased in intensity toward the dentino-enamel junction and which terminated there. The enamel showed no band but only the two gradients in the autographic pattern.

Radio-autographs of newborn rats injected with radio-phosphorus

The molars of animals which received P^{32} within 12 hours of birth (referred to as "newborn") showed an autographic reaction with two gradients, the radial and basal-coronal. At no time did the enamel show a reaction as intense or greater than dentine.

The incisors showed two gradients in the autographic reaction, as described above, but over and above this, a band of reaction just before the dentino-enamel junction in the dentin was observed in the tip of the incisors. This band was present at 6 days after injection, but not three days later.

Radio-autographs of newborn rats injected with radio-calcium

The radio-autographs of newborn rats injected with Ca^{45} were similar to those described above. The molars showed no band of reaction in the dentine while the incisors showed a band in the dentine which, by the ninth day, was no longer present. The enamel in molars and incisors showed only the two types of gradients.

DISCUSSION

The autographic reaction which appeared in the dentine may be ascribed to the formation of new crystals at that particular time when the radio-element was administered. The incisors which erupt at an earlier time than molars would, therefore, have a thicker dentine and enamel, since their development is ahead of that of molars. Therefore, incisors of animals injected at birth show a band of reaction, whereas molars do not, thus indicating that incisors had sufficient matrix already formed for

calcification. For animals injected at the age of 3 days, an autographic reaction band is observed both in the incisors and molars, both of which have at the time of injection at least the minimal amounts of dentine and enamel matrix in which the radio-elements, and hence mineral elements, may be deposited.

The enamel, which is known to be more heavily calcified than dentine (Leicester, 1949), may be thought at first hand to contain a greater amount of radio-active calcium or phosphorus. However, radio-autographs up to 15 days of age, show the heavier reaction over the dentine, which can only be interpreted to mean that the mineral constituents of teeth enter the dentine in greater amounts than the enamel. (This may be reversed in later life.)

The similarities in the fate of the radio-active band in the teeth of new born rats and of those three days old suggests a possibility that some physico-chemical force may be in operation in these structures. Some force which causes a local solution and crystallization of the mineral elements of dentine may well account for the loss of the band of reaction in incisors and molars. It is interesting to note that this loss occurs about 9 days after injection into animals in the very young age group.

Work is continuing on the finer aspects of the fate of the radio-autographic band in dentine.

CONCLUSIONS

1. The mineral metabolism of radio-phosphorus and calcium are identical in teeth.

2. The deposition of these elements is in the form of layers, which appear as bands in radio-autographs.

3. The displacement and change in intensity of the bands (first seen by Bélanger) were confirmed. It is suggested that the direct effect of mechanical factors (pressure and tension) is responsible for these changes.

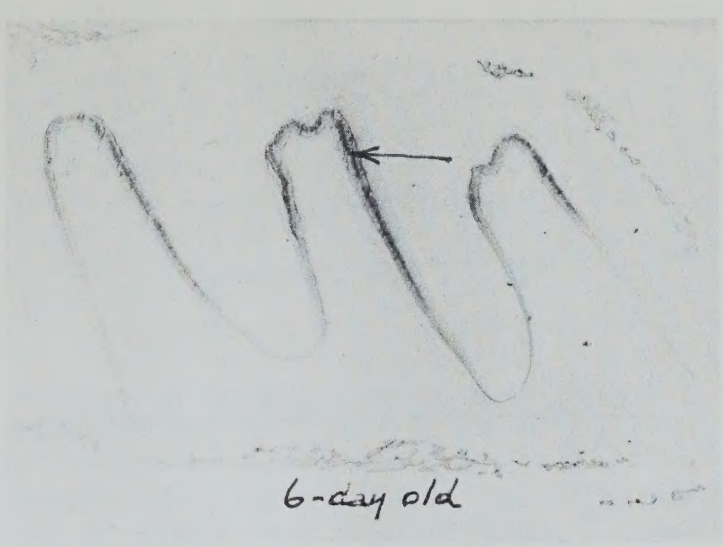

Fig. 1: Photograph of the radio-autograph of the first molar of a rat which received a tracer dose of radio-calcium when 3 days old and sacrificed when 6 days old. Note the band of reaction in the dentine midway between the pulp and the dentino-enamel junction (see arrow).

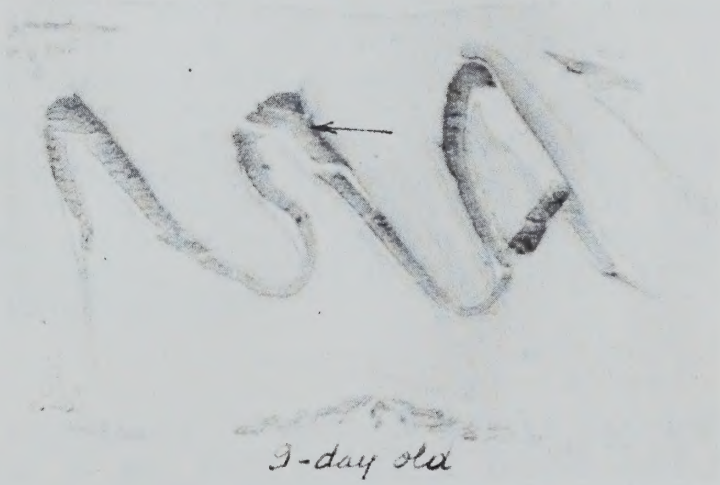

Fig. 2: Radio-autograph of the first molar of a rat given a tracer dose of radio-calcium at birth and sacrificed when 9 days old. Note the reaction in the dentine is maximal at the dentino-enamel junction (see arrow).

APPENDIX "B"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

February 1953

by

Richard C. Greulich

Project No: DR 23

ENTRY OF RADIO-CARBON INTO TEETH

In a previous report (September, 1952) two experiments were announced, the purpose of which was to elucidate, 1) the nature of the materials in dentine and enamel which fix C^{14} derived from labeled sodium bicarbonate; 2) the fate of these materials once formed; and 3) the effects of age upon the pattern of C^{14} fixation in enamel and dentine. These experiments are now well underway; and significant results are available.

METHODS

Experiment I

Each of 12 one-day old rats received 30 μc of C^{14} - labeled sodium bicarbonate. The animals were then sacrificed in pairs at 1, 3, 6, 9, 12, and 15 days after injection. Those animals sacrificed at 9, 12, and 15 days received a second injection of 30 μc two days after the first injection. Hard tissues were fixed in alcohol-formol. The hard tissues of one animal of each pair were then decalcified in formate-citrate. Paraffin sections were prepared for radio-autography. Sections of both decalcified and undecalcified hard tissues were either left unstained or were stained with hematoxylin-eosin, periodic acid-fuchsin sulfurous acid (PA-FSA) or safranin. Some of the hematoxylin-eosin stained sections were treated with ribonuclease or hyaluronidase prior to staining, to remove ribonucleic acid or chondroitin sulfuric acid respectively. Some of the PA-FSA stained sections were treated with beta-amylase prior to staining to remove glycogen. A total of 360 slides were used for histological study and radio-autography.

Experiment II

In the second experiment, a total of 9 rats was employed, 3 of which weighed approximately 30 gm each (weanling rats), 3 of which weighed approximately 100 gm each (young rats), and the remaining 3 of which weighed approximately 260 gm each (adult rats). Each of these animals was given a single subcutaneous injection of C^{14} - labeled sodium bicarbonate which amounted to 80 μc in the adult and young rats, and 40 μc in the weanling rats. Two animals of each weight were killed at 24 hours after injection; the remaining one of each weight was sacrificed 72 hours after injection.

One upper incisor and one humerus was removed from each animal and was fixed in alcohol-formol for 24 hours. The incisor and humerus from each of the animals were placed together in separate cheesecloth bags prior to decalcification. Those incisors and humeri removed at 72 hours after injection, and one set of those removed at 24 hours, were decalcified for 6 days at the top of 100 cc column of a 28% aqueous solution of tetrasodium ethylene diamine tetracetate[x] maintained at a temperature of 45°C. When freshly prepared, the pH of this material was 9.05; after decalcification of bone samples weighing 1 gm taken from adult ra , the pH changed to 9.3.

The remaining sets of incisors and humeri removed at 24 hours after injection were decalcified in the formate-citrate mixture of Morse for 4 days at room temperature. The formate-citrate solution was renewed daily.

The incisors and humeri thus decalcified were then dehydrated in dioxane, embedded in paraffin, and sectioned at 6 μ for histological and radio-autographic study. Sections for histological study were stained with hematoxylin-eosin or PA-FSA, while sections for radio-autography were left unstained or were stained with hematoxylin-eosin or PA-FSA. Sections were also stained by the von Kossa technique to assess the completeness of the decalcification procedures. Radio-autographs were prepared by the coating technique.

In addition to the above, two tibiae, two femora, one humerus, the entire mandible, the maxillary molars of both sides, and the remaining maxillary incisor were removed from each animal, freed of connective tissue and muscle and fixed for 24 hours in alcohol-formol. After fixation, the bones and teeth were washed in running water for 6 hours, dried at 45°C overnight, and weighed. Following this, all of the bones and teeth from each animal were placed in separate cheesecloth bags. The maxillary molars and the single maxillary incisor were freed of alveolar bone insofar as was possible, but the mandibular teeth were freed only of obviously excessive amounts of bone. Thus, the teeth samples were contaminated to some degree by bone.

Decalcification of these samples proceeded according to the plan stated above; that is, teeth and bones from animals sacrificed 72 hours and from one of the animals sacrificed 24 hours after injection were decalcified in ethylene diamine, while the bone and teeth samples from the remaining 24 hour animal were decalcified in formate-citrate. The technique employed here was the same as stated above, except that separate columns of 80 cc ethylene diamine were used for each individual sample of bones or teeth. Similarly, separate containers of formate-citrate were employed.

Following decalcification, the bone and teeth samples were washed in 3 changes of distilled water totalling 20 cc over a period of 6 hours. The washing fluid was then added to the original decalcifying media. After washing, the samples were dried overnight at 45°C and reweighed. The samples were then rehydrated in distilled water at 45°C for 4 hours, after

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x "Sequestrene", obtained from the Alrose Chemical Co., Providence, R.I. in powder form. This material was made up in solution to the desired strength with the aid of heat, and was filtered before use.

which the bones were freed of bone marrow. In the case of teeth, it was found impractical to attempt the removal of marrow and pulp, both from a technical point of view, and also because it was felt that the marrow or pulp did not constitute a significant portion of the weight of the samples.

When the marrow was removed, the bone samples were washed briefly in distilled water, and then along with the teeth, were redried at 45°C overnight. Following this, they were again weighed.

For determination of C^{14} content, the decalcifying media and washing fluid were made up to a known volume (100 cc), and aliquots of 0.5 cc volume were prepared and dried for counting. The organic residues were digested in 2N and NaOH to yield a concentration of 50 mg matrix per cc alkali. An aliquot of 0.5 cc of this digest was prepared for counting. All counts were made in a Tracerlab SC-16 windowless flow counter coupled with a Berkeley decimal scaler (model 1000). Samples of ethylene diamine decalcification media and of the organic matrices resulting from ethylene diamine decalcification were counted for a long enough time to reduce the probable counting error to 2.7%. These samples were of infinite thickness, as were those of organic matrix resulting from formate-citrate decalcification. Samples of formate-citrate decalcifying media were of infinite thinness, but contained no C^{14}. The probable counting error of formate-citrate samples of organic matrix was 8.8%.

RESULTS

Experiment I

Since beta-particle energy of C^{14} is very low, and since the absolute quantity of C^{14} present in a tissue section is minute, very long exposure times (up to a year) are necessary to yield satisfactory radio-autographs. Radio-autographs of the tissues from this experiment were prepared for exposure in the latter part of August, 1952, and at monthly intervals since that time, test autographs have been developed and examined to determine the progress of the radio-autographic reaction. It is only during the past month that radio-autographic exposures have become sufficiently intense for microscopic interpretation. One of the test preparations developed recently was the radio-autograph of a section of an unstained and decalcified lower incisor from an animal which had been sacrificed on day 9 after administration on day one of 30 μc of C^{14} - labeled sodium bicarbonate, and readministration of 30 μc on day 3. This reaction was not sufficiently intense to be recorded photographically for illustration. However, it was possible to make an accurate drawing of the shape of the section and to indicate diagrammatically the distribution of the black silver grains indicative of the presence of C^{14}. This drawing is represented in Fig. 1. Reference to this figure reveals that the radio-autographic reaction predominates in the dentine, particularly toward the distal end of the tooth. In addition, it is possible to identify the dual origin of the dentinal reaction as two discrete lines of silver grains which diverge one from the other until, in the region of the crown, they are separated by a considerable distance. Presumably, the outermost line of reaction resulted from the first injection of C^{14} on day one, while the inner line arose as a consequence of the re-injection on day 3. It was not possible to draw any conclusions as to the C^{14} uptake

in enamel matrix or in any of the dental tissues near the root of the incisor due to the relatively low level of radiation provided by these structures during the 4-month interval of autographic exposure.

Experiment II

Only the analytical data is available at present. The radio-autographic preparations are still undergoing exposure. It should be mentioned, however, that von Kossa stained sections similar to those used for radio-autography did not show any evidence of the presence of bone salts, thus indicating that decalcification, both in ethylene diamine and in formate-citrate, had gone to completion. Since the bone and tooth samples used for estimation of C^{14} content were decalcified in a similar manner, it is presumed that in these also, decalcification was complete.

The pertinent data from C^{14} analysis in bones and teeth of weanling, young, and adult rats is presented in Table 1.

Comparison of columns 8 and 9 of Table 1, which indicate the percentage of the weights in grams constituted by the inorganic and organic fractions of each tooth or bone sample show that formate-citrate decalcification consistently removed less inorganic material than did similar treatment with ethylene diamine (EDTA), and conversely, with but one exception, tended to leave more weight in the organic residue. Another interesting observation was that the percentage composition of the tooth samples is apparently already stabilized even in the 30 gm animals, since it does not change appreciably in young or adult animals. On the other hand, the percentage composition of bone in terms of organic and inorganic materials is altered markedly with increasing body weight. Thus, while the composition of teeth samples remains in the approximate ratio of 3:1 inorganic: organic, regardless of age, that of the bones varies from 1:1 to 2:1 with increasing age, never attaining the high inorganic: organic ratio of the teeth. This observation is in agreement with the findings of many other investigators.

Columns 10 and 11 of Table 1 indicate the total recorded C^{14} activity in the entire weight of each bone or tooth sample. In column 10, with but one exception, no counts were recorded. Since the counts of the inorganic fraction were made directly from the decalcifying media, it was not surprising that formate-citrate residues contained no counts. The acid pH of this material precludes the possibility of inorganic carbonates being held by it.

On the other hand, the pH of ethylene diamine was sufficiently alkaline (pH = 9.3 after decalcification of a 1 gm sample of bone from animal number 2 of the 260 gm weight group) to retain carbonates in solution. It is felt that the one positive result which was obtained (496.5 counts/100 sec.) bears witness to this fact. Since plates for counting were made under conditions of "infinite thickness", self absorption within the sample could account for the lack of countable radiation; that is, some radio-activity could be present in the sample which was not recorded. However, plates of organic matrix were also prepared in "infinite thickness", and since self absorption is relatively independent of the nature of the absorbing material at "infinite thickness", a comparison of the data from

ethylene diamine samples in columns 10 and 11 is entirely valid. Hence, it is seen that by far the greatest part of C^{14} activity in bones and teeth resides in the organic matrix. Very little activity appears in the inorganic fractions. These findings are in disagreement with those of other workers, and it is suggested that this may be due to the fact that the methods employed by other investigators for removing the inorganic material from hard tissues in determinations of this type are far more stringent than those employed here, and may perhaps remove as well a "labile" carbon component of the organic matrix.

Columns 12 and 13 simply present the data of columns 10 and 11 in terms of the activity in counts per 100 seconds per gram of inorganic or organic material. Since the youngest animals (30 gm group) received only half the injected dose administered to the two older groups, it is assumed that had they received twice the initial dose, twice the amount of activity would have been recovered. This assumption is probably true because the actual weight of bicarbonate administered was insignificant, i.e., the dose was at a "tracer" level. Appearing in parentheses below the actual recorded values from the 30 gm group are the values which probably would have been obtained had these animals received the same level of radio-activity as did the older animals.

Perhaps the most striking feature of columns 12 and 13 (also 10 and 11) is the rapid removal of organically bound C^{14} from the adult bones and teeth. Thus, the organic matrix of the teeth of a 260 gm animal sacrificed 24 hours after injection contain some 2,500 counts/100 sec./gm matrix, while in an animal treated in an identical manner but sacrificed 72 hours later, no counts remain. This phenomenon becomes less evident with decreasing age, so that in the teeth of weanling animals little if any activity is lost over the 72-hour period. Similarly, in the bone samples, the activity falls from 2,302.9 counts/100 sec./gm matrix at 24 hours to zero counts at 72 hours in the adult rats, while in the weanling rats, there is a small decrease in the C^{14} content.

Those substances in the organic matrices of bones and teeth which are responsible for C^{14} activity are of an unknown composition. However, the processing techniques through which the samples were taken before C^{14} assay, i.e., alcohol-formol fixation, decalcification in hot acidic or alkaline solutions, and washing in hot water, probably remove most of the low-molecular-weight compounds which are soluble in hot water, dilute acidic solutions or dilute alkaline solutions. Some compounds of high molecular weight would be removed as well, such as water soluble proteins and glycogen (soluble in hot water).

Thus, the substances remaining in the organic matrix at the time of C^{14} assay were probably composed of proteins, carbohydrates other than glycogen, protein-carbohydrate complexes (mucoproteins), and finally, some lipoidal materials.

Work will continue to determine the significance of these data and the chemical nature of the radio-active products.

CONCLUSION

Previous investigations have demonstrated that C^{14} - labeled sodium bicarbonate injected into very young rats could be incorporated into the organic matrices of enamel, dentine and bone. The original evidence for this suggestion was that hematoxylin-stained preparations of teeth and bones gave striking radio-autographic reactions in these structures, even though it was demonstrated that the acidity of the hematoxylin solution employed was sufficient to remove all of the inorganic material present.

Subsequent investigation of the radio-autographic reactions of unstained sections of teeth or bones from very young rats injected with C^{14} bicarbonate, indicated that little diminution of the radio-autographic images was obtained even after decalcification in 5% nitric acid, thus tending to support the concept of preferential C^{14} -bicarbonate fixation in organic matrix in very young animals.

This report deals primarily with the results obtained from a study of the effects of age on C^{14} -bicarbonate fixation in organic matrix. The results indicate that there is a preferential incorporation of the isotopic material in the organic matrix rather than the inorganic fraction of bones and teeth. In fact, little if any C^{14} -bicarbonate appears in the inorganic fractions. C^{14} uptake by organic matrix is more marked in the youngest animals (30 gm), and reaches a minimum in adult animals (260 gm).

Finally, the C^{14} -bicarbonate is incorporated into the organic matrix of teeth and bones of adult animals is turned over very rapidly. Thus, while significant amounts of C^{14} were found in adult matrix at 24 hours after administration of the isotope, none was found at 72 hours. Young (110 gm) and weanling (30 gm) rats did not exhibit such a marked turnover phenomenon; in fact, in weanling animals, little if any decrease in the amount of C^{14} present in organic matrix occurred during the 72-hour period. Radio-autographic studies of this phenomenon are in progress.

(1) SAMPLE	(2) AVERAGE WT. OF ANIMAL g.	(3) ANIMAL NO.	(4) TIME SACRIFICED hr.	(5) DECALCIFICATION MEDIUM	(6) WT. INORGANIC FRACTION g.	(7) WT. ORGANIC FRACTION g.	(8) % WT. INORGANIC	(9) % WT. ORGANIC	(10) COUNT/100SEC. INORGANIC	(11) COUNT/100SEC. ORGANIC	(12) COUNT/100SEC./GM. INORGANIC	(13) COUNT/100SEC./GM. ORGANIC
TEETH	260 (adult)	1.	24	Fo-Ci	0.7151	0.2393	73.5	24.7	0.0	437.0	0.0	1,825.0
		2.	24	EDTA	0.6170	0.1762	78.5	22.4	0.0	446.6	0.0	2,534.6
		3.	72	EDTA	0.6503	0.1907	78.0	22.8	0.0	0.0	0.0	0.0
	110 (young)	1.	24	Fo-Ci	0.3005	0.1043	73.5	25.5	0.0	133.7[x]	0.0	1,280.0[x]
		2.	24	EDTA	0.3687	0.1136	77.0	23.7	0.0	370.8	0.0	3,264.1
		3.	72	EDTA	0.3811	0.1239	76.0	24.7	0.0	297.0	0.0	2,397.1
	30 (weanling)	1.	24	Fo-Ci	0.0867	0.0372	69.5	29.8	0.0	892.0 (1,784.0)	0.0	23,980.0 (47,960.0)
		2.	24	EDTA	0.0850	0.0332	72.5	28.2	0.0	466.4 (932.8)	0.0	14,048.2 (28,096.4)
		3.	72	EDTA	0.1364	0.0483	74.5	26.3	0.0	694.4 (1,388.8)	0.0	14,376.8 (28,753.6)
BONES	260 (adult)	1.	24	Fo-Ci	1.2813	0.6790	61.0	32.3	0.0	1,303.0	0.0	1,920.0
		2.	24	EDTA	1.3406	0.6738	64.5	32.4	496.5	1,551.7	370.3	2,302.9
		3.	72	EDTA	1.2970	0.6315	65.0	31.7	0.0	0.0	0.0	0.0
	110 (young)	1.	24	Fo-Ci	0.4360	0.3201	53.5	39.4	0.0	2,275.0	0.0	7,100.0
		2.	24	EDTA	0.5512	0.3285	60.5	32.3	0.0	1,362.4	0.0	4,147.3
		3.	72	EDTA	0.4826	0.2763	61.0	36.2	0.0	697.4	0.0	2,524.1
	30 (weanling)	1.	24	Fo-Ci	0.0755	0.1232	34.5	56.2	0.0	2,458.0 (4,916.0)	0.0	19,930.0 (39,860.0)
		2.	24	EDTA	0.0895	0.1009	44.0	49.6	0.0	1,576.0 (3,152.0	0.0	15,627.0 (31,254.0)
		3.	72	EDTA	0.1079	0.1141	46.0	48.9	0.0	1,247.5 (2,495.0)	0.0	10,933.4 (21,866.8)

Fo-Ci - Formate-citrate; EDTA - ethylene diamine tetracetate; [x]sample partially lost.

Table I.
(See text for explanation)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
SAMPLE	AVERAGE WT. OF ANIMAL g.	ANI-MAL NO.	TIME SACRI-FICED hr.	DECALCI-FICATION MEDIUM	Fe-Cr ATOM RATIO	Mg. Fe/100 g. ORGANIC MATTER	Mg. Fe/100 g. DRY TISSUE	Mg. Fe/100 g. ASH	Mg. Fe/100 g. ORGANIC MATTER	Mg. Fe/100 g. DRY TISSUE	Mg. Fe/100 g. ASH	Mg. Fe/100 g. ORGANIC MATTER
BONES	110 (young)	1.	24	EDTA	Fe-Cr	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		2.	24	EDTA	Fe-Cr	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		3.	72	EDTA	Fe-Cr	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	30 (weanling)	1.	24	EDTA	Fe-Cr	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		2.	24	EDTA	Fe-Cr	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		3.	72	EDTA	Fe-Cr	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TEETH	110 (young)	1.	24	EDTA	Fe-Cr	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		2.	24	EDTA	Fe-Cr	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		3.	72	EDTA	Fe-Cr	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	30 (weanling)	1.	24	EDTA	Fe-Cr	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		2.	24	EDTA	Fe-Cr	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		3.	72	EDTA	Fe-Cr	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table II. Fe-Cr complex as a chelating agent - EDTA - Formic-Sodium Formate Buffer pH 4.2

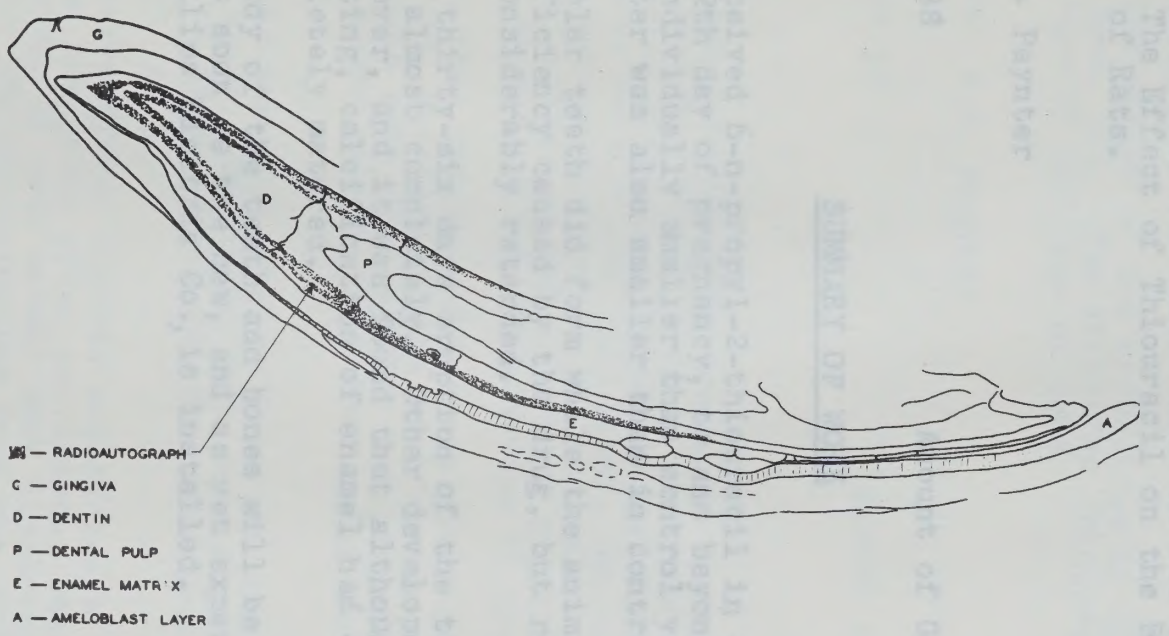
G
D
P
E
A
— RADIOAUTOGRAPH
C — GINGIVA
D — DENTIN
P — DENTAL PULP
E — ENAMEL MATRIX
A — AMELOBLAST LAYER

Fig. 1

Стр. 7

APPENDIX "C"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

Subject: The Effect of the Endocrines on the Teeth and Jaws. II. The Effect of Thiouracil on the Bone of the Jaws of Rats.

Grantee: K.J. Paynter

Project No. DR 38 Amount of Grant: $2610.

SUMMARY OF WORK

Rats which received 6-n-propyl-2-thiouracil in the drinking water from the 10th or 12th day of pregnancy, to and beyond term, delivered young which were individually smaller than control young. The number of animals per litter was also smaller than in control litters.

Each of the molar teeth did form while the animal was under the thyroid hormone deficiency caused by the drug, but rate of development of the teeth was considerably retarded.

By the age of thirty-six days eruption of the teeth appeared to have been arrested almost completely. Other developmental processes had continued, however, and it was found that although the third molars were far from erupting, calcification of enamel had continued and this structure was completely matured.

Soft X-ray study of the teeth and bones will be made in the very near future as soon as the new, and as yet experimental, apparatus loaned to us by Philips Electric Co., is installed.

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Subject: The effect of the endocrines on the teeth and jaws. II. The effect of thiouracil on the bone of the jaws of rats

Grantee: Dr. K.J. Paynter

Project No.: DR 38 Amount of Grant: $2610

As was mentioned in the last report to the committee, it was felt that following a study of teeth and jaws of rats which received 6-n-propyl-2-thiouracil subcutaneously from birth, it would be of interest to continue this study, but to administer the drug earlier, before the lamina for the molar teeth invaginated. Thus one might see whether or not a lack of thyroid hormone had any effect on the embryological organization of the dental tissues.

Accordingly, five pregnant rats were given thiouracil in their drinking water, starting on the twelfth day of pregnancy. Litters born to these rats were smaller in numbers than normal controls, and the young weighed less than young born to untreated controls.

Mortality rate was high, but to date ten specimens have been prepared for study. The young were sacrificed at various ages from one day to thirty-six days, two specimens having lived to this latter age.

Heads, pituitary gland, thyroid and tibias of each animal were removed for study after sacrifice. One half the mandible and one tibia of each animal were dissected out for radiographic study. The corresponding part was sectioned and stained in hematoxylin and erythrosin.

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REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

Subject: The effect of the endocrines on the teeth and jaws. II. The effect of thiouracil on the bone of the jaws of rats

Grantee: Dr. K.J. Payster

Project No.: DR 34

Amount of Grant: $2510

Due to lack of grenz ray equipment we have not been able to make the radiographic study which was intended. Within the next few days, however, we are to have installed an apparatus which, it is believed will produce much the same result as a grenz ray apparatus. Correspondence through Philips Electric Company with their parent company, N.V. Philips Gloeilampenfabreiken, Eindhoven, Nederlands, has resulted in the loan for six months by this company of a "KT" X-ray apparatus which uses voltage as low as 10 KV DC and a berylium-window tube to produce relatively soft radiation. It is hoped that this apparatus will serve our purpose.

Once the X-ray pictures have been made, measurements will be made of the mandibular teeth of the animals to determine whether any change in tooth size results from propylthiouracil administration at this early age.

Preliminary study of the sections of the teeth indicating lack of thyroid hormone does not prevent the formation of the teeth. Each of the molar laminae does invaginate to form the individual teeth. Rate of development was retarded as it was in those animals which received the propylthiouracil subcutaneously after birth. It appeared, however, that in the older specimens eruption was more retarded than was calcification and maturation of enamel. The third molar had not erupted by the thirty-sixth day, yet complete maturation of enamel had occurred. In the controls the reverse is generally the case, i.e. eruption occurs before the enamel at the cemento-enamel junction is completely matured.

The bone of the jaw histologically appeared very dense. The lamellae were heavy and thick with reduced marrow space between them.

As a control study, to ascertain whether or not effects produced by propylthiouracil administration are due to the drug rather than to lack of thyroid hormone, further work is now under way in which pregnant rats will receive propylthiouracil in the drinking water, and will also receive thyroxine in optimal amounts. Thus any changes or toxic effects observed could be attributed to the drug rather than to a lack of thyroid hormone.

The amount of money being applied for this year in connection with this study is less than in the past due to the fact that it will not be necessary to pay a technician from this grant during the year. The services of a technician who is being paid from other sources have been made available to me.

Due to lack of grens ray equipment we have not been able to make the radiographic study which was intended. Within the next few days, however, we are to have installed an apparatus which, it is believed will produce much the same result as a grens ray apparatus. Correspondence through Philips Electric Company with their parent company, N.V. Philips Gloeilampenfabrieken, Eindhoven, Nederlands, has resulted in the loan for six months by this company of a "KT" X-ray apparatus which uses voltage as low as 10 KV DC and a beryllium-window tube to produce relatively soft radiation. It is hoped that this apparatus will serve our purpose.

Once the X-ray pictures have been made, measurements will be made of the mandibular teeth of the animals to determine whether any change in tooth size results from propylthiouracil administration at this early age.

Preliminary study of the sections of the teeth indicating lack of thyroid hormone does not prevent the formation of the teeth. Each of the molar lamina does invaginate to form the individual teeth. Rate of development was retarded as it was in those animals which received the propylthiouracil subcutaneously after birth. It appeared, however, that in the older specimens eruption was more retarded than was calcification and maturation of enamel. The third molar had not erupted by the thirty-sixth day, yet complete maturation of enamel had occurred. In the controls the reverse is generally the case, i.e. eruption occurs before the enamel at the cemento-enamel junction is completely matured.

The bone of the jaw histologically appeared very dense. The lamellae were heavy and thick with reduced marrow space between them.

As a control study, to ascertain whether or not effects produced by propylthiouracil administration are due to the drug rather than to lack of thyroid hormone, further work is now under way in which pregnant rats will receive propylthiouracil in the drinking water, and will also receive thyroxine in optimal amounts. Thus any changes or toxic effects observed could be attributed to the drug rather than to a lack of thyroid hormone.

The amount of money being applied for this year in connection with this study is less than in the past due to the fact that it will not be necessary to pay a technician from this grant during the year. The services of a technician who is being paid from other sources have been made available to me.

APPENDIX "D"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

Subject: EXPERIMENTAL FUSOSPIROCHETAL INFECTION WITH MIXTURES OF PURE CULTURES

Grantee: J.B. Macdonald

Project No.: DR 35 Amount of Grant: $3,300

SUMMARY OF WORK

The experimental infection designated HG, which has been transmitted serially through guinea pigs, has been analyzed. 113 isolations have been attempted, 30 of which were successful. The organisms isolated included several strains of streptococci, strains of Bacteroides, strains of motile Gram-negative rods, one strain of Fusobacterium, strains of Gram-positive non-motile rods and a culture of Treponema microdentium. Of these all were anaerobic with the exception of one strain of streptococci and one strain of Gram-positive rods, both of which were facultative.

Strains isolated were examined by a series of morphological cultural and biochemical tests in order to determine replication. Results of these tests indicated that 14 of the 30 strains were duplicates of other isolations. There has been thus isolated in pure culture 16 distinctive organisms.

These are being recombined in various combinations and inoculated into normal and scorbutic guinea pigs and guinea pigs prepared by previous inoculation with Scillaren B.

APPENDIX "D"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

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Grantee: J.B. Macdonald

Project No.: DR 35 Amount of Grant: $3,300

SUMMARY OF WORK

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Strains isolated were examined by a series of morphological cultural and biochemical tests in order to determine replication. Results of these tests indicated that 1/4 of the 30 strains were duplicates of other isolations. There has been thus isolated in pure culture 16 distinctive organisms.

These are being recombined in various combinations and inoculated into normal and scorbutic guinea pigs and guinea pigs prepared by previous inoculation with Bacillaren B.

APPENDIX "D"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

Subject: Experimental fusospirochetal infection with mixtures of pure cultures

Grantee: Dr. J.B. Macdonald

Project No.: DR 35 Amount of Grant: $3,300

Analysis of Guinea Pig Fusospirochetal Exudate

The experimental infection designated HG and initiated in guinea pigs two years ago has been exhaustively analyzed in preparation for recombination experiments now under way.

One hundred and thirteen isolations have been attempted by a variety of techniques and using a variety of media. Thirty isolations have been successful.

Media

The media used have been referred to in earlier reports. Those found most useful were:

1. A veal heart infusion has been described by Proske and Sayers (1934) prepared as a blood agar with 10% defibrinated sheep blood.

2. A spirochete medium prepared from the Proske Sayers base by addition of agar, ascitic fluid, neutralized cysteine HCl and either fresh guinea pig kidney or an aqueous extract prepared from guinea pig kidney.

3. A fluid medium of the same composition as No. 2 but without sugar.

4. Difco thioglycollate broth.

Methods

The following methods were successful in isolating organisms from the exudate.

1. Streaked blood agar incubated anaerobically from 5 days to 2 weeks.

2. Subculture to blood agar or other media from the migrating edge of confluent growth on blood agar.

3. Inoculation of Noguchi tubes from the spirochete haze surrounding mixed growth in spirochete cup plates or on blood agar plates.

Cultures isolated were examined morphologically, culturally and by a series of biochemical tests to determine replication. (See tables I and II).

Morphologic examination

Colonies on blood agar were examined by artificial light using a stereoscopic microscope at 10 X magnification. The colony appearance on primary isolation and repeated subculture was recorded. In several instances it was found that initial appearance and subsequent appearance of colonies was dissimilar.

Microscopic appearance was determined by dark field examination and by Gram stain.

Cultural examination

Growth was examined in respect to rate, amount and appearance in Difco thioglycollate and in veal heart infusion broth with ascitic fluid and kidney extract (PSAFKE broth).

Biochemical tests

Fermentation power was tested by growth in a sugar-free thioglycollate broth prepared by Baltimore Biological Laboratories (BBL broth) to which was added respectively 1% dextrose, sucrose or mannite. The media were titrated to pH 7.2. The pH of cultures of the various organisms in these media was determined with a glass electrode potentiometer. Cultures were examined for acid production in litmus milk and the solubility in 10% sodium hydroxide of the clot, if any, was tested. Production of hydrogen sulphide was tested in peptone iron agar tubes made by mixing equal parts of Difco thioglycollate agar (BX agar) and Difco peptone iron agar. Formation of indol was tested in BX broth culture by shaking 1 ml. of xylol with the culture, heating to 55°C. and adding 7 or 8 drops of Ehrlich reagent. A positive test was indicated by the development of a pink colour. Reduction of nitrate was tested in BBL thioglycollate broth containing 0.1% potassium nitrate. Presence of nitrite was tested by the method of Topley and Wilson (1945), using an acid solution of sulfanilamide (0.5 gm. sulfanilamide in 150 ml. of 0.05 N. HCl acid), 10% aqueous ammonium sulfamate, and an acid solution of alpha naphthylamine (1 gm. dissolved in 22 ml. of water by heating, and then filtered into 180 ml. of dilute acetic acid). Two drops of the sulfanilamide solution were added to the culture followed in 3 minutes by two drops of ammonium sulfamate and two drops of alpha naphthylamine. The development of a pink or red colour indicated a positive test.

Positive controls using a culture of E. coli were used in testing for indol and for hydrogen sulphide. The nitrite test was assessed quantitatively by the addition of sodium nitrite to BBl broth. It was found that approximately 0.0001% sodium nitrite gave a faintly positive test. Negative controls of all tests were run on sterile broth. Inoculation in all cases was by capillary pipette using 5 to 10 drops depending on the turbidity of the seed culture. Peptone iron agar tubes were inoculated by stabbing the solidified medium.

Results

By the above methods (applied to all except spirochete cultures) replication was disclosed in 14 instances. This left a total of 16 cultures isolated from the HG guinea pig exudate (Table I). Seven of the strains were Gram-positive cocci. All of these were strict anaerobes with the exception of one which was facultative (JSI). The latter was a non-hemolysing streptococcus. Two strains of Gram-positive rods were isolated. One of these (JB3) was a strict anaerobe while the other (JR3) was facultative. This latter organism along with the facultative JS1 constituted the only aerobic organisms in the exudate.

Three strains of anaerobic Gram-negative non-motile rods with rounded ends (presumably Bacteroides) were isolated. One of these (K110) was identified as the black pigment-producing Bacteroides nigrescens (formerly B. melinogenicum). Colonies of this organism were subcultured from plates streaked with exudate many times without obtaining growth. It was observed that colonies of this organism appeared to be always in close proximity to a small low convex smooth grey colony which was composed of Gram-positive cocci. One ml. of a Seitz-filtrate of a broth culture of this latter organism was added to 5 ml. of thioglycollate broth in an effort to facilitate growth of the Bacteroides. This proved to be successful and it has been possible to maintain the Bacteroides strain through many subcultures in this way. The strain also develops hemolysing satellite colonies around colonies of the streptococcus when the two are cross-streaked.

Only one strain of Fusobacterium was isolated. This was a tapered rod 6 - 20 microns long appearing singly or in tandem formation. Colonies were low convex, mottled and exhibited a peculiar pink and green surface irridescence. This organism conformed in its characteristics to the description of Fusobacterium given by Bøe (1941).

Two strains of motile rods were isolated. One (JV1) belonged with group I of the motile rods described in earlier reports and has been named Vibrio sputorum. The second was a Gram-negative feebly motile rod with rounded ends which tended in dark fields or stained preparations to form rosette arrangements. Its characteristics differ from those of the 3 groups as described earlier.

The spirochetes were considered pure only in the sense that they were separated from other bacterial forms. They belonged in the group designated on morphologic criteria as Treponema microdentium.

Recombination experiments are in progress in which various groups of pure cultures isolated from HG exudate are being inoculated into normal and scorbutic guinea pigs and into guinea pigs prepared by previous inoculation with scillaren B.

TABLE I. MORPHOLOGY OF PURE CULTURES FROM HG

Strain	Original Colony	Colony after S.C.	Darkfield
JS1	smooth, pinpoint translucent, convex	smooth, pinpoint grey, convex	cocci-singles and pairs
JR4	smooth, < 1 mm. translucent, flat umbonate	smooth, pinpoint grey, convex	cocci-singles, pairs and short chains
JR5	smooth, pinpoint grey, convex	smooth, pinpoint grey, convex	cocci in clusters
K98	lacy, ½ mm. white	smooth, < ½ mm. convex, cream	cocci in short chains and clusters
K100	smooth, < ½ mm. convex, white	smooth, ½ mm. convex, grey	cocci in short chains
K112	smooth, ½ mm. convex, pink irregular outline	smooth, ½ mm. convex, pink round, surrounded by crater	cocci in chains
JS9	smooth, pinpoint translucent, convex	smooth, ½ mm. semi-translucent grey-white	cocci in clusters
JR3	rough, ½ mm. yellow, low convex	smooth, ½ mm. cream, umbonate	rods pleomorphic 4-30 microns
JB3	smooth, < ½ mm. semi-translucent low convex	smooth, ½ mm. cream, convex	rods 2-4 microns
JR6	smooth, < ½ mm. translucent convex	smooth, < ½ mm. translucent convex	ovoid rods singles occasionally pairs
K92	smooth, 1 mm. cream, convex fimbriate	smooth, 2 mm. cream, convex fimbriate, greening	rods 1-15 microns irregular rounded ends
K110	smooth, ½ mm. black, convex	smooth, ½ mm. yellow to black convex	rods pleomorphic 1-6 microns

TABLE I. MORPHOLOGY OF PURE CULTURES FROM HG (con.)

Strain	Original Colony	Colony after S.C.	Darkfield
JF5	smooth, ½ mm. grey, flat	smooth, ½ mm. mottled grey low convex	rods 6-8 microns tapered
JV5	rough, ½ mm. grey, low convex greening	smooth, ½ mm. grey, low convex fimbriate greening	rods 2 microns straight or curved darting motility
K80	2 mm., grey flat, mottled irregular	smooth, 1 mm. low convex mottled round, irridescent	rods 6-8 microns sluggish motility

TABLE II. CULTURAL AND BIOCHEMICAL CHARACTERISTICS OF PURE CULTURES FROM HG

Strain	Rate of Growth	B236	PSAFKE	Gram	Sugars D.	Sugars S.	Sugars M.	H_2S	Indol	Litmus	Nitrate
JS1	1 day A 2 days N	+++ floc.	++ turb.	pos.	4.3	4.4	7.2	neg.	neg.	acid c clot	neg.
JR4	2 days	+++ floc.	+ fine gran. ppt.	pos.	4.9	6.9	6.9	neg.	+ -	sl. acid	+
JR5	2 days	+++ floc.	+ gran. sed.	pos.	5.6	6.9	5.5	neg.	+	sl. acid	+
K98	3 days	++ floc.	+ turb. ++ floc. sed.	pos.	6.4	6.62	6.9	neg.	neg.	neg.	neg.
K100	3 days	++ floc. & coarse gran.	+++ floc. sed.	pos.	6.8	6.8	6.9	neg.	neg.	neg.	neg.
K112	3 days	++ floc.	+ floc.	pos.	5.7	6.7	7.0	neg.	neg.	neg.	neg.
JS9	2 days	+++ floc.	+ adherent sed.	pos.	5.4	7.25	7.2	neg.	+	neg.	++
JR3	2 days N or A	++ floc.	+ turb.	pos.	4.6	4.6	6.8	neg.	neg.	acid c clot	neg.
JB3	2 days	+++ floc.	++ gran. sed.	pos.	4.7	7.0	7.1	neg.	+ -	acid	+ -
JR6	3 days	+ turb.	+ floc. sed.	neg.	n.g.	n.g.	n.g.	neg.	neg.	acid	n.g.
K92	3 days	+ floc.	+ floc. sed.	neg. c gram-pos. gran.	6.15	6.8	6.85	neg.	+ -	neg.	neg.
K110	7 days	(with JS9 filtrate + turb.	n.g.	neg.	n.g.	+ growth	n.g.	n.g.	N O T T E S T E D		

n.g. No growth

TABLE II. CULTURAL AND BIOCHEMICAL CHARACTERISTICS OF PURE CULTURES FROM HG (con.)

Strain	Rate of Growth	B236	PSAFKE	Gram	D.	S.	M.	H_2S	Indol	Litmus	Nitrate
JF5	5 days	++ floc.	+ adherent floc. sed.	neg.	6.1	6.1	6.8	neg.	+	acid	neg.
JV5	5 days	± floc.	± turb.	neg.	7.0	7.1	7.2	+	neg.	neg.	neg.
K80	5 days	++ floc.	± turb. - ++ sed.	neg.	6.5	6.8	7.1	n.g.	+	neg.	± -

n.g. No growth

APPENDIX "E"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

Subject: Chemical Mechanism in Dental Caries.

Grantee: J.J. Rae

Project No.: DR 1 Amount of Grant: $1080

SUMMARY OF WORK

(1) Ammonia production in saliva is a function of the pH. The inhibitory effect of glucose on ammonia production seems to be a pH effect. The stimulating effect of glucose on ammonia production in the presence of urea is also probably due to the ability of glucose to maintain the pH at a maximum for ammonia production for a longer time.

(2) Lactic acid production is also closely related to the pH of the medium. Low pH's (4.0) inhibit lactic acid production but pH's of about 8.0 seem to exert no inhibitory effect.

(3) The inhibitory substance obtained by treating ground tooth enamel with bromoform has not been identified. A summary of our work to date on this subject is presented.

Proposed work includes the further study of:- the effect of urea and salts on ammonia production; the effect of urea and salts on lactic acid production and the identification of the inhibitory substance.

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APPENDIX "E"

ANNUAL REPORT

(Interim Report No.15)

Subject: Chemical Mechanisms in Dental Caries.

Grantee: J.J. Rae

Project: DR 1

The work covered under this project during the last year can be conveniently divided into three parts.

Sec. 1. Ammonia Production in Saliva.

Sec. 2. Lactic Acid Production.

Sec. 3. A Substance in Tooth Enamel Which Inhibits the Growth of l.b.a.

Sec. (1) Ammonia Production in Saliva.

As a result of Kesel's[3] research, in which he stated that ammonia has an inhibitory effect on l.b.a. growth and acid production, a great many ammonia and urea containing dentifrices appeared on the market. If his results are valid one would expect to find more ammonia in caries-free saliva. In consulting with the literature it was found that there were wide differences of opinion as to whether caries-free saliva did or did not contain more ammonia than saliva from caries-immune persons.

Since the ammonia content of saliva is very low we decided to measure instead the ammonia produced after 24 hours incubation at 37°. When this was done we found no positive correlation between ammonia production and l.b.a. count.

When urea is added to saliva the ammonia produced in 24 hours is increased 5-6 times showing the presence of an active urease in saliva.

The addition of glucose to the saliva inhibited ammonia production but the addition of glucose to the saliva-urea mixture enhanced ammonia production.

The preliminary work on this project was done by Mr. Clegg and the later work by Miss Ballantyne. It had reached the above stage by the summer of 1951.

In October 1952 Miss Shemilt was assigned the problem of finding answers to these 4 questions:

APPENDIX "B"

ANNUAL REPORT

(Interim Report No.15)

Subject: Chemical Mechanisms in Dental Caries.

Grantee: J.J. Rae

Project: DR 1

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In October 1952 Miss Shemilt was assigned the problem of finding answers to these 4 questions:

(1) What research has been done on ammonia in saliva since 1950?

(2) Are there any better methods for determining ammonia?

(3) Why does glucose inhibit ammonia production in saliva?

(4) Why does glucose enhance ammonia production in saliva-urea mixtures?

(1) Weisberger[1] had done work similar to ours and had obtained comparable results, i.e., incubation of saliva produces 2 to 4 times the amount of urea plus ammonia nitrogen originally present and that glucose reduces the increase to a significant 15%. The increase was almost entirely in the NH_3 fraction. He concluded that glucose favours proteolysis over the process of deamination. Since he found the presence of glucose on incubating saliva promotes the liberation of free amino acids (proteolysis) from substances which contain them in a combined form while deaminization reactions are very definitely decreased in rate if they were not actually halted. On the other hand when saliva is incubated without added glucose, deaminization reactions are favoured.

Jenkins and Wright[2] were also unable to substantiate Kesel's[3] work. They observed the effect of added urea in increasing ammonia production. In the discussion of their results they stated that any salt at an alkaline pH will inhibit lactate production and that the ammonium ion had no specific inhibitory action. They also demonstrated that although high concentrations of ammonium or sodium salts (5%) inhibited acid production lower concentrations (1%) increased acid production.

(2) A comparison was made between the formol method for the determination of ammonia nitrogen and Permutit method which had been used in this work by Clegg and Ballantyne[4]. The initial results failed to satisfy us that this method was a reliable one for determining NH_3-N. This conclusion was reached when standard solutions of ammonium sulfate, urea, d,l-alanine, ammonium oxalate and ammonium acetate gave results which were neither in agreement with the calculated ones, nor reproducible. Work on this project will be continued, as it is felt that the results obtained are not complete and that other factors bear investigating.

(3) When one attempts to answer the question why glucose inhibits ammonia production the first approach would be to investigate the effect of pH since the tubes containing glucose after 24 hours had a pH about 4.0 whereas those tubes without glucose had a pH about 8.0.

The inhibitory effect of glucose (2%) was again observed.

A series of buffers was then prepared at pH 4.0, 5.0, 6.0, 7.0, and 8.0 and the ammonia production measured at these pH's.

The results are shown in Table 1.

(1) What research has been done on ammonia in saliva since 1950?

(2) Are there any better methods for determining ammonia?

(3) Why does glucose inhibit ammonia production in saliva?

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(3) When one attempts to answer the question why glucose inhibits ammonia production the first approach would be to investigate the effect of pH since the tubes containing glucose after 2½ hours had a pH about 4.0 whereas those tubes without glucose had a pH about 6.0.

The inhibitory effect of glucose (2%) was again observed.

A series of buffers was then prepared at pH 4.0, 5.0, 6.0, 7.0, and 8.0 and the ammonia production measured at these pH's.

The results are shown in Table 1.

TABLE 1

pH		mg % NH_3 22 hrs at 37°
Before	After	
4.17	4.12	1.4
5.12	5.12	1.7
5.95	6.37	17.7
6.72	6.80	21.7
7.80	7.62	20.0
7.31	7.51	21.6

These results indicate that the pH of the solution is a determining factor in NH_3 production. The glucose effect can then be explained in the following way. Glucose acting as a substrate for acid-producing bacteria in the saliva would provide the source of acid which lowers the pH and thus inhibits ammonia production.

The fact that glucose itself does not completely inhibit ammonia production is shown by the table below.

TABLE II

The Effect of pH and Glucose on Ammonia Production

Tube Content	mg NH_3 24 hrs at 37° 0 = 100	Final pH
Saliva + glucose + buffer (6.0)	310	5.1
Saliva + glucose + buffer (8.0)	215	6.1
Saliva + buffer (8.0)	665	7.6
Saliva (control)	835	6.8

TABLE I

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The saliva samples were incubated with glucose at a final concentration of 2%, as before. 80% of the solutions consisted of veronal-acetate buffer at pH's of 6 and 8. Controls were incubated, using no glucose. One control contained 80% buffer, at pH of 8.0 plus saliva. The other consisted of only water and saliva.

Samples were analyzed for ammonia content at 0, 4 1/2, and 9 1/2 hours of incubation. Another series was set up, and samples were analyzed at 0, 18, and 24 hours.

Series C and D (no glucose added) show the usual trend we have observed before. The ammonia concentration rises quite steadily as incubation proceeds. The pH in series D fluctuates around pH 7.0. In other experiments, at higher concentrations of saliva, the pH generally rose somewhat.

In series A and B (glucose added), the pH drop which always occurs when glucose is present, is evident. Due to the high buffer concentration this drop is now as steep as that which occurs with solutions not so highly buffered.

The trend in the results is quite evident. Comparing series A with series C and D, it may be observed that glucose provides some inhibition to ammonia production. Series B indicates that the inhibition is more marked at the higher pH.

Tests were made to find out if the veronal-acetate buffer interfered, and the work showed it did not. However, these tests were made with solutions containing only 50% buffer. Perhaps the higher buffer concentration causes a slight amount of reduction in the amount of NH_3 produced due to a salt effect. This work is being continued. NOTE TO SECTION I - See page 8.

Sec. (2) Lactic Acid Production in Saliva

The widely held view that dental caries is initiated by acid decalcification postulates that lactic acid is produced by bacterial action on food residues in the mouth. This has led to the use of l.b.a. counts as a measure of caries susceptibility. The determination of l.b.a. counts is tedious and time consuming so that it was thought that a rapid chemical method for the determination of lactic acid as a criterion of caries susceptibility would be desirable.

EXPERIMENTAL

Saliva was incubated in a buffered solution at pH 5 with 1% glucose (final volume) at 37° for one hour. Lactic acid was determined by the colorimetric method proposed by Barker and Summerson[5]. The results (Table I) showed there was no relationship between l.b.a. count and lactic acid production under these conditions. Even zero count salivas showed significant lactic acid production.

TABLE I

Lactic Acid Production and l.b.a. Counts in Saliva.

Tube No	l.b.a. Count	Lactic Acid mg % in 1 hr at 37°
1	0	45.7
2	0	41.2
3	21,000	16.7
4	30,000	61.0
5	60,000	20.6
6	200,000	19.1
7	200,000	62.0
8	300,000	45.0

Jenkins and Wright[2] have shown that the inhibitory effect on lactic acid production of high concentrations of salts (5%) which produce an alkaline reaction, such as sodium phosphate and ammonium phosphate, was due to the high pH produced. The ammonium ion had no specific inhibitory effect as had been suggested by Kesel[3]. Low concentrations (0.5%) of the same salts enhanced lactic acid production. This is probably due to the fact that these salts maintain the pH at an optimum for lactic acid production for a longer time. They showed that alkalinity itself did not inhibit lactic acid production by incubating saliva and glucose and maintaining the pH at 8 by small additions of sodium hydroxide solution at frequent intervals.

In view of these findings, it was decided to investigate the effect of buffered solutions at pH 4 and 8 on lactic acid production. The results obtained showed that at a pH of 4.0 there was no lactic acid produced but that at a pH of 8.0 there was apparently no inhibition of lactic acid production. At a pH of 8.0 a correlation was observed between l.b.a. count and lactic acid production, as shown in Table II.

TABLE II

l.b.a. Counts and Lactic Acid Production in Saliva at pH 8.0

Tube No	l.b.a. Count	mg % Lactic Acid (pH 8.0)
1	0	13.5
2	0	180
3	0	99
4	50	24.8
5	15,000	189
6	75,000	475

TABLE I

Lactic Acid Production and I.b.a. Counts in Saliva.

Tube No	I.b.a. Count	Lactic Acid mg % in 1 hr at 37°
1	0	45.7
2	0	41.2
3	21,000	16.7
4	30,000	81.0
5	60,000	20.6
6	200,000	19.1
7	200,000	62.0
8	300,000	45.0

Jenkins and Wright[2] have shown that the inhibitory effect on lactic acid production of high concentrations of salts (5%) which produce an alkaline reaction, such as sodium phosphate and ammonium phosphate, was due to the high pH produced. The ammonium ion had no specific inhibitory effect as had been suggested by Kessel[3]. Low concentrations (0.5%) of the same salts enhanced lactic acid production. This is probably due to the fact that these salts maintain the pH at an optimum for lactic acid production for a longer time. They showed that alkalinity itself did not inhibit lactic acid production by incubating saliva and glucose and maintaining the pH at 8 by small additions of sodium hydroxide solution at frequent intervals.

In view of these findings, it was decided to investigate the effect of buffered solutions at pH 4 and 8 on lactic acid production. The results obtained showed that at a pH of 4.0 there was no lactic acid produced but that at a pH of 8.0 there was apparently no inhibition of lactic acid production. At a pH of 8.0 a correlation was observed between I.b.a. count and lactic acid production, as shown in Table II.

TABLE II

I.b.a. Counts and Lactic Acid Production in Saliva at pH 8.0

Tube No	I.b.a. Count	mg % Lactic Acid (pH 8.0)
1	0	13.5
2	0	160
3	0	99
4	50	24.8
5	15,000	189
6	75,000	475

In the light of these findings it was decided to determine the optimum pH for lactic acid production for saliva and to study the effect of urea and salts on the lactic acid production as observed by Jenkins and Wright[2].

Sec. (3) A Summary of Research on an Inhibiting Substance for l.b.a. Growth Developed When Ground Human Tooth Enamel is Shaken With Bromoform.

The finding of Wakeman and Smith[6] that ground teeth gave bacterial counts ten times greater than whole teeth prompted us to investigate the effect of adding ground tooth enamel to culture media. We found that it inhibited the growth of l.b.a.

Ground calcium phosphate did not have an inhibitory effect on l.b.a.

Autoclaving the ground tooth substance did not destroy its inhibitory power. The ground tooth enamel used in these experiments was prepared by the Manley and Hodge[7] bromoform separation and the fact that freshly ground whole teeth had no such effect made us suspect that bromoform was causing the observed inhibition. However dentine prepared by the Manley and Hodge[7] method showed no inhibition neither did $Ca_3(PO_4)_2$ or bone meal treated with bromoform. Bromoform itself showed no inhibition when 0.05 ml. was added to 4 ml. of l.b.a. culture yet treated ground tooth enamel showed inhibition in amounts as little as 10 mg. even after heating to 160° (B.P. bromoform 149°). Chloroform when substituted for bromoform yielded a tooth enamel which had no inhibitory effect.

Extracting the bromoform treated tooth enamel with water destroyed its inhibiting action. The aqueous extract also showed no inhibition. However another treatment with bromoform gave an inhibitory ground tooth enamel.

The bromoform after shaking with tooth enamel was evaporated to dryness and this residue showed no inhibitory powers.

Extraction of treated tooth enamel with alcohol produced an extract which on evaporation gave 19.3 mg. of an inhibitory substance. This material was soluble in ether and acetone and by qualitative tests was found to contain no sulfur, phosphorous or nitrogen. A positive test for the presence of chloride was obtained.

Attempts to purify the treated enamel showed that the inhibiting substance could be extracted with 70% methanol.

An alcoholic extract after evaporation to dryness yielded a chloroform soluble and chloroform insoluble portion. The former fraction yielded crystals on evaporation with no inhibitory powers.

The addition of silver nitrate to remove the chloride from 5 ml. of an alcoholic extract (22 mg. of solids per ml.) of bromoform-treated enamel produced a precipitate (269 mg.). The chloride-free filtrate was still found to be inhibitory. To this was added hydrochloric acid to precipitate excess silver. No precipitate was produced. The solution was then neutralized and was still found to have an inhibitory effect. Work is being continued on this aspect of the work.

Sec. (3) A Summary of Research on an Inhibiting Substance for I.b.s.

Growth Developed When Ground Human Tooth Enamel is Shaken With Bromoform.

The finding of Wakeman and Smith[6] that ground teeth gave bacterial counts ten times greater than whole teeth prompted us to investigate the effect of adding ground tooth enamel to culture media. We found that it inhibited the growth of I.b.s.

Ground calcium phosphate did not have an inhibitory effect on I.b.s.

Autoclaving the ground tooth substance did not destroy its inhibitory power. The ground tooth enamel used in these experiments was prepared by the Manly and Hodge[7] bromoform separation and the fact that freshly ground whole teeth had no such effect made us suspect that bromoform was causing the observed inhibition. However dentine prepared by the Manly and Hodge[7] method showed no inhibition neither did $Ca_3(PO_4)_2$ or bone meal treated with bromoform. Bromoform itself showed no inhibition when 0.05 ml. was added to 4 ml. of I.b.s. culture yet treated ground tooth enamel showed inhibition in amounts as little as 10 mg. even after heating to 160° (B.P. bromoform 149°). Chloroform when substituted for bromoform yielded a tooth enamel which had no inhibitory effect.

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The addition of silver nitrate to remove the chloride from 5 ml. of an alcoholic extract (22 mg. of solids per ml.) of bromoform-treated enamel produced a precipitate (209 mg.). The chloride-free filtrate was still found to be inhibitory. To this was added hydrochloric acid to precipitate excess silver. No precipitate was produced. The solution was then neutralized and was still found to have an inhibitory effect. Work is being continued on this aspect of the work.

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2. Jenkins and Wright, Brit. Dent. Jour., 90, 127, 1951.

3. Kesel, Am. J. Ortho & Oral. Surg., 33, 68, 1947

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Ibid., 31, 281, 1952.

5. Barker and Summerson, J.B.C., 138, 535, 1941.

6. Wakeman and Smith, J.D.R., 27, 41, 1948.

7. Manley and Hodge, J.D.R., 18, 133, 1939.

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1. Weisberger, J.D.R. 25, 49, 1946.

2. Jenkins and Wright, Brit. Dent. Jour., 90, 127, 1951.

3. Kessel, Am. J. Ortho & Oral. Surg., 33, 68, 1947

4. Clegg and Ballantyne, J.D.R., 30, 385, 1951.
Ibid., 31, 283, 1952.

5. Barker and Summerson, J.B.C., 138, 535, 1941.

6. Wakeman and Smith, J.D.R., 27, 41, 1948.

7. Manley and Hodge, J.D.R., 18, 133, 1939.

NOTE TO SECTION I

An address was given 29 December 1952 by Dr. J.J. Rae on "Ammonia Production in Saliva" at the American Association for the Advancement of Science Symposium: Chemistry and Dentistry Team Up for Progress.

For reference purposes, a copy of this paper is on file in the office of the Secretary of the Associate Committee on Dental Research. Following is the author's summary of his address:-

SUMMARY

1. The use of ammoniated dentifrices and urea-containing tooth powders seems to be based on a rather insecure foundation, since ammonium salts have been shown to have no specific inhibitory effect on acid production.

2. It has not been proved that saliva from caries-free individuals contains or produces more ammonia than saliva from caries-active persons. On the basis of our evidence there is no relation between ammonia production of saliva and the degree of dental caries.

3. The ability of saliva to convert urea to ammonia has been demonstrated but again no direct relation has been observed between urease activity and the degree of caries in an individual.

4. High concentrations of urea and either sodium or ammonium salts do inhibit lactic acid production but lower concentrations stimulate acid production in saliva-glucose mixtures.

5. More information is needed about the cause and progress of dental caries before any pronouncement can be made about the efficacy of any suggested treatment. However, if we subscribe to the bacteria-acid theory it seems unlikely that ammonium salts exert any specific inhibitory effect apart from the fact that they act as buffers.

6. On the other hand the ammonia producing mechanisms of salivary bacteria should repay further study. If they should be a responsible factor in maintaining neutrality in the saliva the indiscriminate addition of enzyme inhibitors to saliva such as fluoride and penicillin might be of doubtful value since such inhibitors might destroy the ability to produce ammonia.

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APPENDIX "F"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

Subject: A histopathological study of the effects of a potassium deficiency and of a pyridoxine deficiency on the teeth and surrounding structures of dogs

Grantee: Dr. A.G. Racey

Project No.: DR 39 Amount of Grant: $1600

SUMMARY OF WORK

While much has been written about dietary deficiencies, one should remember that this does not only mean the lack of a proper diet, but also includes dietary intake, absorption and utilization of the food factors. This study deals with the lack of potassium and pyridoxine in the diet. Since these deficiencies are of interest to the physician also, one must not think that the teeth and supporting structures alone are involved, but that the deficiency may affect the body as a whole. Accordingly, medical co-operation is essential for the proper treatment of dietary deficiencies when first noticed by the dentist.

The dogs for the deficiency studies were obtained from the Department of Experimental Surgery of McGill University. These dogs were as young as possible in order to survive the major operations done by the Surgery Department.

The part that potassium plays in the normal metabolism of the body has been studied extensively with respect to electrolytic balance, nutrition, toxicity, growth, etc. There has also been extensive clinical studies of the effects of potassium in many clinical conditions. One is referred to the review by W.O. Fenn: The Role of Potassium in Physiological Process, Physiological Review, Vol. 20, p. 377, 1940.

The potassium deficiency was obtained by a synthetic diet devised by Drs. D. Currie and R. Forse of the Department of Experimental Surgery. This diet contained the necessary proteins, carbohydrates and fats. A salt mixture was used which contained the necessary minerals and the potassium element was substituted for calcium. Only those vitamins were used which have been proven essential for normal growth and maintenance.

For a complete history of the dogs on which the potassium deficiency was studied, one is referred to the thesis by Dr. D.M. Currie, The Effect of Potassium Deficiency on Gastric Secretion, McGill University Thesis for M.Sc. (Surgery).

APPENDIX "B"

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March, 1952

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In 1933, Webster and Armour showed that the vitamin B complex was essential for the normal production of gastric secretion; and, in 1944 Street, Cowgill and Zimmerman obtained evidence that pyridoxine was essential for the normal production of gastric secretion. On the basis of the above works it was decided by Dr. R. Forse to investigate the effect of pyridoxine deficiency on gastric deficiency. It was from these experimental animals that the material was obtained for the deficiency study on the teeth and surrounding structures. The pyridoxine deficient state was obtained by means of a synthetic diet and was accelerated by the use of desoxypyridoxine.

For a complete history of the dogs one is referred to "The Effect of Pyridoxine Deficiency on Gastric Secretion" R.A.Forse, B.Sc., M.D., C.M., McGill University Thesis M.Sc.(Surgery).

Procedure

The material was preserved in 10% formalin and was cut into pieces suitable for decalcification and sectioning of the desirable teeth. It was found that better fixation of the pulp was obtained if an opening was made into the pulp chamber with a small burr and allowed more rapid penetration of the formalin.

Decalcification of the teeth and mandible was carried out by means of an ionic decalcifier and by several acid solutions. The ionic decalcifier was excellent when studying the pulp alone, but if there were soft tissues present the gases released tended to disturb the tissues and tear it. It did not completely decalcify the mandible and teeth of the dogs without extensive destruction of the tissues.

The acid solutions were found to be much better because of the lack of damage to the tissues, but the time of decalcification was long. The end point of decalcification was determined as far as possible by the use of a sharp pin and by X-rays.

The following results were obtained from the deficiency studies and were compared with a control animal on a normal diet. It may be noted here that the potassium deficient animal seemed to be the easiest to section on the microtome, then the normal and pyridoxine.

Epithelium

Normal: highly keratinized stratified squamous epithelium with deep papillae and it took the stain very well.

Potassium Deficiency: much similar to the normal except for a lesser amount of keratinization.

Pyridoxine Deficiency: thin and desquamating, did not stain very well and in a process of degeneration. In the submucosal area, a chronic inflammation was present.

Periodontal Membrane

Normal: consists of dense collagen fibres, fibrocytes and blood vessels.

Potassium Deficiency: the fibres are not as well orientated and they appear to lack definition.

Pyridoxine Deficiency: a greater number of blood vessels present and some evidence of edema.

Alveolar Bone

Normal: dense and dark staining

Potassium Deficiency: evidence of osteoporosis and vacuolated areas containing a reticular network, less microscopic evidence of attachment of the periodontal fibres.

Pyridoxine Deficiency: evidence of osteoporosis.

Dental Pulp

Normal: consists of fibrocytes, odontoblasts and blood vessels.

Potassium Deficiency: appears to be a slight hyperemia.

Pyridoxine Deficiency: extensive hyperemia and the vessels are engorged with red blood cells. There is a lack of fibrocytes and the predentine appears to be absent. The odontoblasts appear to be in a process of degeneration.

COMMENTS

It may be noted from the above results that the potassium deficient showed very little change from the normal. This may be due to the fact that potassium is present extensively in the tissues of the body and may not be depleted while on a potassium deficient diet. It is felt that more drastic changes would be shown if the animal was raised on a potassium deficient diet.

In the pyridoxine deficient animal, the most drastic changes appear to have taken place in the epithelium and the dental pulp. This may be of importance in periodontal disease because of the

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In the pyridoxine deficient animal, the most drastic changes appear to have taken place in the epithelium and the dental pulp. This may be of importance in periodontal disease because of the

protective role which epithelium plays. For if there is a chronic inflammation present, then it is much more difficult to treat if one does not realize that the deficiency is contributing to it.

The lack of keratinization would also contribute to periodontal disease because of the lack of protection of the oral cavity from infection.

The pyridoxine deficiency may also be of importance in dental caries and restorative dentistry. For if secondary dentine is not laid down as a protective reaction under very deep caries, one is more liable to have an exposure of the dental pulp and subsequent loss of the tooth. This is also of great importance when a large restoration has to be placed in a tooth. These people would then fail to have the necessary resistance to deep caries as a person on a normal diet.

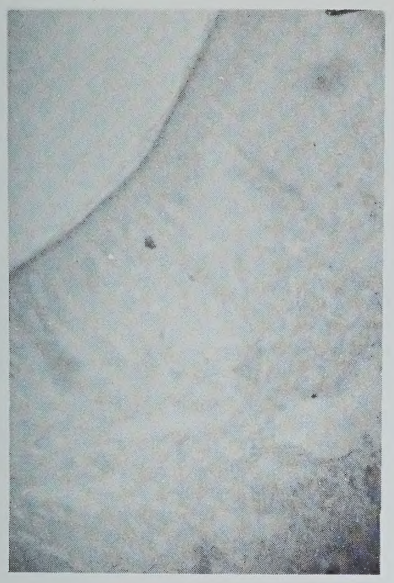

Fig. 1 Normal

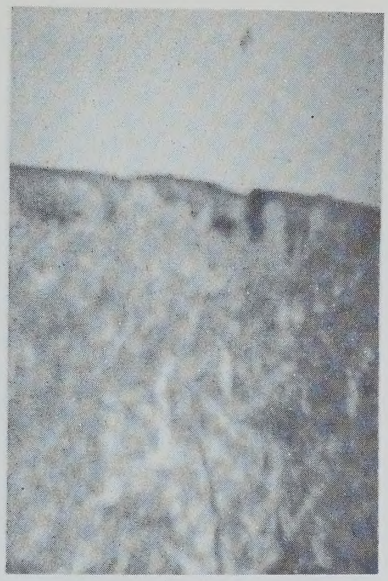

Fig. 2 Potassium

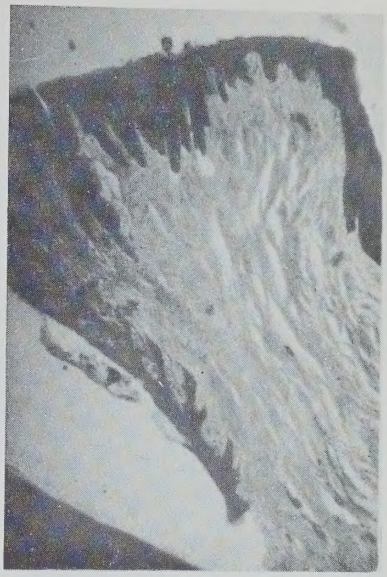

Fig. 3 Pyridoxine

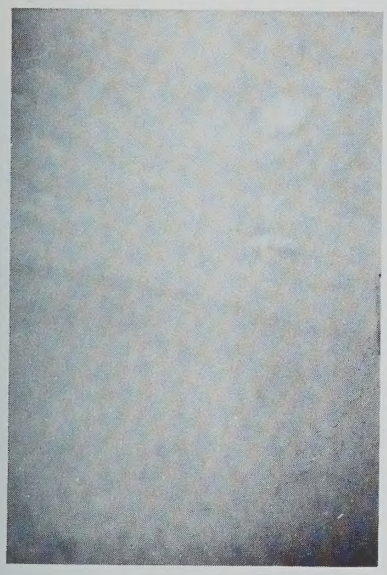

Fig. 4 Normal

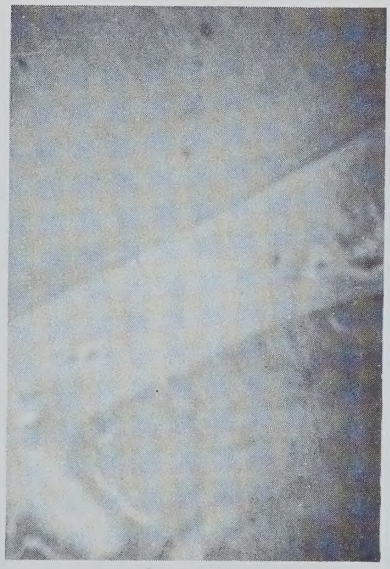

Fig. 5 Potassium L.P.

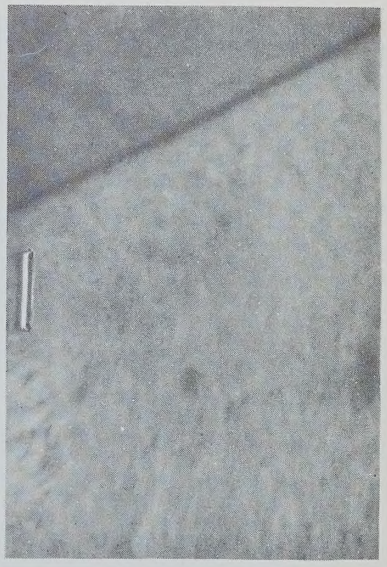

Fig. 6 Potassium H.P.

Fig. 7 Pyridoxine

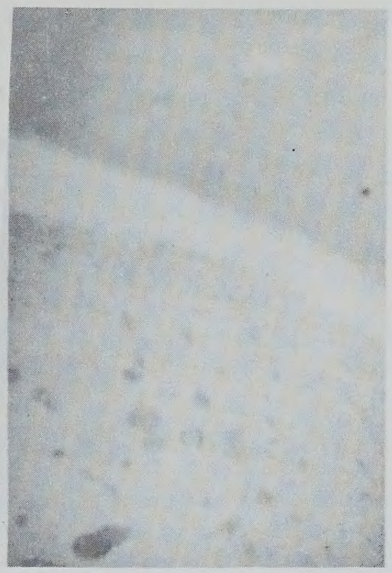

Fig. 8 Normal L.P.

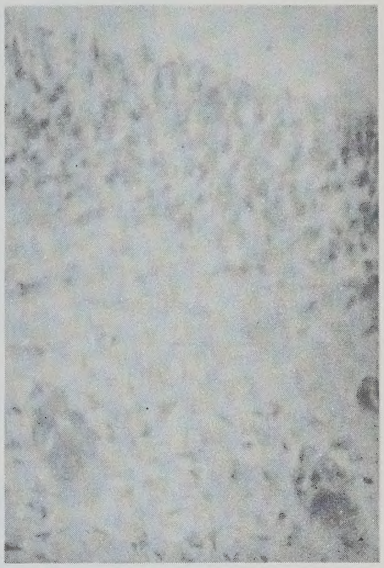

Fig. 9 Normal H.P.

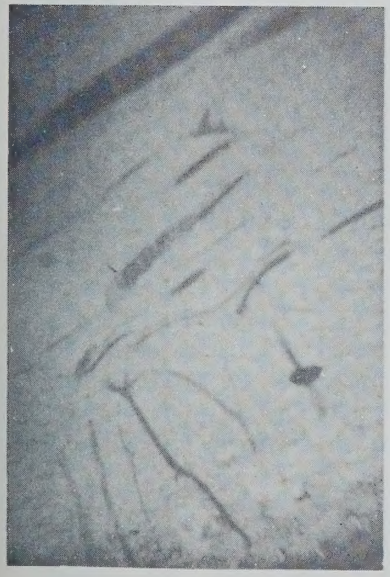

Fig. 10 Potassium L.P.

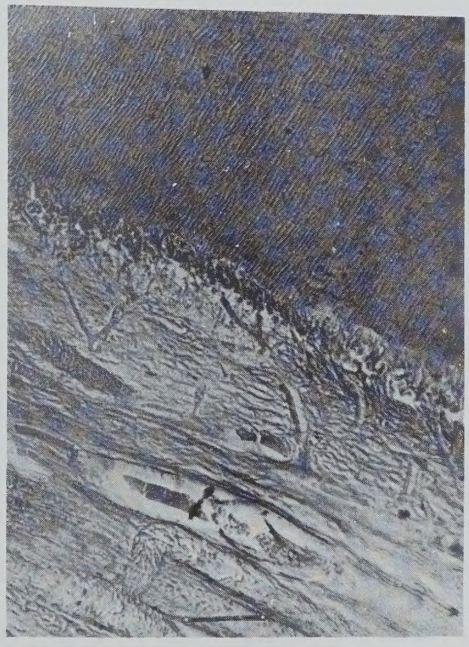

Fig. 11 Pyridoxine L.P.

Fig. 12 Pyridoxine H.P.

APPENDIX "G"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

Subject: An investigation of the mechanism of repair of the supporting structures of the teeth.

Grantee: Dr. Charles H. Best

Project No.: DR 22 Amount of Grant: $4,200

SUMMARY OF WORK

In 1949, Goldman defined reattachment as a regrowth of the attachment apparatus after treatment of a periodontal pocket. A true reattachment, then, necessitates a new growth of cementum and alveolar process and the establishment of a functional periodontal membrane. This definition of reattachment will be used in this report except where there is reattachment by means of a new growth of cementum without the corresponding regrowth of the alveolar process. This type of repair will be referred to as cemental reattachment.

Following the presentation of the paper on reattachment after the production of epithelialized pockets, the evidence was again thoroughly reviewed. Certain changes in the interpretation of the findings were made and a paper, study 3, in this series, was prepared for publication. However, it was considered necessary to further check the method for producing the epithelialized pockets.

Accordingly, the same procedures for the production of epithelialized pockets in study 3, were repeated on 24 cuspid teeth. In this study the animals were sacrificed with healing times ranging from 9 to 33 days, instead of approximately 20 months, as in study 3. The results of this study did not invalid any of the conclusions from study 3. The findings are reported in more detail in the longer report. However, the desirability of developing another method of preparing epithelialized pockets for subsequent studies was indicated.

To date, in this investigation, it appears that for re-attachment, osteogenic cells from the bony margins of the wound must be induced to proliferate and grow around the denuded root.

Summary of work (cont'd)

In studies 1 and 2, in this series, this appears to have occurred following the organization of a blood clot, in study 3 by granulation. The experimental conditions in studies 1 and 2 differed from the clinical conditions in periodontal disease in two important aspects. Therefore, further study is required before the clinical application of this type of repair can be made. This problem is discussed and the progress so far is reported in the longer report.

Attempts have been made to produce epithelialized intrabony pockets in dogs, 5 cases. Considerable difficulty has been encountered with these preparations as the bony shelf tends to resorb before epitheliazation is completed.

Two therapeutic approaches to the treatment of periodontal disease are widely advocated, i.e., the so-called conservative and radical treatments. To a degree these methods are conflicting. In view of this, it is desirable to ascertain which type of therapy favours reattachment by granulation. However, before this study can be started, another method of preparing epithelialized pockets must be developed where small amounts of reattachment can be accurately detected. It is not feasible to keep animals 20 months as in study 3. Three methods are being tried at present.

The results of the study of the nature of the growth process in these tissues and the effect of certain hormones in the investigation described in the interim report are discussed in the longer report. As a result of this study, an improved method for routinely preparing sections of the three molar teeth in the correct plane was indicated. The progress so far is reported.

APPENDIX "G"

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Reattachment has been defined as a growth of the attachment apparatus of the teeth after treatment of a periodontal pocket. Reattachment, therefore, necessitates a new growth of cementum and bone with incorporation of periodontal fibres and the establishment of a functional periodontal membrane. In this report this interpretation of reattachment will be used, except when referring to cemental attachment where there is no regeneration of the alveolar process. When referring to this type of repair, the term cemental reattachment will be used.

Following the presentation of the paper at the last annual meeting on regrowth of the attachment apparatus of the teeth, after the production of epithelialized pockets, the evidence was again thoroughly reviewed. Certain changes in interpretation of the findings were made and a paper, study 3 in this series was prepared for publication. However, it was considered necessary to check the method for preventing reattachment until epithelization of the pocket wall occurred. Certain preliminary studies had indicated that epithelization had occurred, but a histologic investigation was deemed necessary to permit a more accurate interpretation of the sections in this and subsequent studies.

Accordingly, the same procedures for the production of epithelialized pockets were repeated on 24 cuspid teeth. In study 3, the animals were kept 19 to 21 months. In this study, the animals were sacrificed with healing times ranging from 9 to 33 days. Blocks of tissue were removed and histologic sections prepared. Briefly, it was found that it took approximately 30 days for epithelization and the formation of an epithelial attachment. In some cases, epithelization occurred slightly apical to the line of surgical attachment. Thus the lower part of the pocket must have been formed by a process other than surgical detachment. In other cases there appeared to be a small amount of reattachment, approximately 1 mm. at the base of the lesion. It was difficult to determine if this reattachment occurred prior to the down growth of epithelium as occurred in studies 1 and 2 of this series, or

following the down growth of epithelium as in study 3. At present it is believed that proliferation of osteogenic cells by granulation occurred prior to the epithelial downgrowth. This would result in reattachment. In any case the validity of study 3 is not affected as the reattachment was less than 2 mm. in all cases where reattachment occurred in this study. These findings coincide with those of Skillin and Lunquist (1936). However, this study did show the desirability of developing a method for producing epithelialized pockets, where the area of the root, exposed by surgical means, could be definitely ascertained in the histological section. Any further detachment then must have come about by a process similar in many respects at least to the detachment and tissue loss occurring in periodontal disease. Also the sequence of events in the reparative process could be more definitely determined if any reattachment was definitely prevented until epithelization of the pocket occurred. The development of such a method for producing periodontal pockets will be referred to later in this report. A paper reporting the above findings may be prepared for publication later if the knowledge acquired has some bearing on the reparative process.

To date, from this investigation, it appears that for reattachment, osteogenic cells from the bony margins of the pocket must be induced to proliferate and grow over the denuded root. In studies 1 and 2, this appeared to have occurred following the organization of a blood clot. In study 3, by granulation. One of the main differences between the experimental conditions in studies 1 and 2, and those encountered clinically in periodontal diseases, is that in the experimental pockets the periosteum from the alveolar process remained on the detached gingival flap. This may be an important factor in the reparative process in these studies. In all probability, reattachment by granulation will have important but limited application clinically. Many times reattachment by organization of a blood clot, as occurred in studies 1 and 2, will be more suitable. It appears necessary therefore to study the role of the periosteum in the healing process occurring in studies 1 and 2. This investigation is now being carried on.

The role of the periosteum in the repair of bone has been a controversial issue for many years. The dispute is whether it has bone forming properties, or if it functions mainly as a limiting membrane preventing the escape of osteoblasts into the surrounding tissues. Preliminary studies, 3 cases, indicate that the alleged osteogenic capacity of the periosteum is not important in this work. Additional work is being carried on to determine if this conclusion is valid. To determine its role as a limiting membrane, efforts were made to obtain a gingival flap without any periosteum on its inner aspect, but in all other aspects, with conditions similar to those as in studies 1 and 2. This would permit comparative studies Efforts to remove the periosteum from the gingival flap surgically, failed. However,

after various trials, the following method was developed that appears to be suitable. A gingival flap was made, the alveolar process was removed exposing the root, the flap was resutured and reattachment was secured as in study 1. After 46 days the flap was again laid back. As the alveolar process was not present, it was hoped that the periosteum had been replaced by gingival connective tissue. Preliminary studies suggested that this was the case. When the gingival flap was again detached, it would differ from the original flap in that there would be no periosteum present on its inner aspect. However, if it was carefully removed, the cementoblast layer would adhere to the inner wall of the detached flap. If the flap was scraped, removing the cementoblasts there would be no osteogenic cells on the flap. Preliminary investigations, 7 cases carried through to sectioning, suggest that by means of this method further light could be thrown on the following important problems.

1. The role of the periosteum in healing of bone.

2. Whether or not proliferating fibroblasts under similar environmental conditions can differentiate to osteoblasts.

3. Waerberg's conception, based on his researches, of the nature of the healing in experimentally produced pockets.

4. The possibility of connective tissue reattachment to the old cementum without cementum formation as suggested by McCall and Box.

The disadvantage of this method is that each case must have uneventful normal healing following two or three operations.

Another indirect method of determining the role of the periosteum in bone healing is being carried out. Epithelialized periodontal pockets are being prepared and left until the periosteum disappears from the detached gingival flap. This differs from the study described above in that the inner surface of the flap is now covered by epithelium. Attempts are being made to induce the osteogenic cells at the bony margins to proliferate and cover the exposed root by supplying what might be described as an artificial periosteum. Several of these cases, using different materials are now in the second healing period. If this principle is established and found to work, applications in orthopedic surgery may be possible.

Attempts have been made in 5 cases to produce an experimental intra-bony pocket in dogs. From our conception of the nature of the reparative process, it seems quite possible that reattachment in these cases could be quickly achieved following the organization of a blood clot as in studies 1 and 2. However, considerable

difficulty has been encountered in preparing intra-bony pockets in the dog, as the bony shelf tends to resorb in the healing process.

In periodontal therapy, two approaches to treatment have widespread recognition, i.e., the so-called conservative and radical methods. Briefly, in the radical method, the pocket is eradicated by the removal of the soft tissue wall by surgical means. In the conservative approach, the soft tissue wall is retained. It seems desirable to ascertain which method produces conditions most favourable for reattachment by granulation. However, before such a study can be started an improved method for the preparing of periodontal pockets must be devised. Enough cases must be prepared and sectioned and the histologic picture studied, so that later even small amounts of reattachment can be detected in the treated cases. It is not practical to keep the animals for 20 months. Three methods for preparing periodontal pockets are being tried at present.

Another phase of this investigation of the mechanism of repair of the supporting structures is a study of the growth process in these tissues. While information regarding the growth of these structures is of definite interest to dentistry, the primary purpose of this investigation is to throw further light on the fundamental problems in repair.

The growth process was studied when accelerated, in the normal, and when inhibited. It is accelerated in young rats by daily injections of growth hormone and inhibited by hypophysectomy[x]. This also permits a study of the effect of these factors on the growth of the supporting structures. It seems probable that the factors that influence the physiologic mechanisms involved in growth would similarly affect those involved in repair.

Forty-seven young rats were used in this study. They were divided in 7 groups for treatment or controls. At the end of the study the jaws were removed and fixed in 10% formol saline. Approximately 600 paraffin sections were made of the molar teeth and supporting structures on one side of the mandible and stained by hematoxylin and eosin.

x This study was done with the co-operation of Dr. R.E. Haist, Department of Physiology.

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* This study was done with the co-operation of Dr. R.E. Beals, Department of Physiology.

GROUP I

H.I.G.

Hypophysectomized rats fed ad lib and injected with 1 mg. of growth hormone per day for 28 days.

Animal	Initial Weight	Final Weight	Gain
H.I.G. 13	112 gms.	206 gms.	94 gms.
14	120 "	182 "	62 "
15	108 "	166 "	58 "
16	100 "	198 "	98 "
17	104 "	170 "	66 "
18	108 "	180 "	72 "
Total	652 "	1102 "	450 "
Average	108 "	183 "	75 "

GROUP 2

P.F.H.I.G. - Normal rats pair-fed with H.I.G.

Animal	Initial Weight	Final Weight	Gain
PFHIG 13	112 gms.	196 gms.	84 gms.
14	120 "	180 "	60 "
15	108 "	178 "	70 "
16	100 "	148 "	48 "
17	102 "	170 "	68 "
18	109 "	130 "	21 "
Total	651 "	1002 "	351 "
Average	108 "	137 "	58 "

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14	120 "	180 "	60 "
15	108 "	178 "	70 "
16	100 "	148 "	48 "
17	102 "	170 "	68 "
18	109 "	130 "	21 "
Total	651 "	1002 "	351 "
Average	108 "	167 "	58 "

GROUP 3

G.H. - Normal rats pair-fed with P.F.G.H. for 28 days and received 1 mg. growth hormone per day for 28 days.

Animal	Initial Weight	Final Weight	Gain
GH 3	110 gms.	206 gms.	96 gms.
4	111 "	208 "	97 "
5	106 "	190 "	84 "
6	132 "	230 "	98 "
7	130 "	270 "	140 "
8	124 "	168 "	44 "
Total	713 "	1272 "	559 "
Average	119 "	212 "	93 "

GROUP 4

P.F.G.H. - Normal rats fed ad lib used as controls for G.H. group.

Animal	Initial Weight	Final Weight	Gain
PFGH 3	112 gms.	173 gms.	61 gms.
4	100 "	208 "	108 "
5	102 "	194 "	92 "
6	132 "	260 "	128 "
7	130 "	235 "	105 "
8	120 "	178 "	58 "
Total	696 "	1248 "	552 "
Average	116 "	208 "	92 "

GROUP 5

C.A.D. - Normal rats fed ad lib for 28 days.

Animal	Initial Weight	Final Weight	Gain
CAD 5	111 gms.	173 gms.	62 gms.
6	98 "	168 "	70 "
7	98 "	148 "	50 "
8	110 "	166 "	56 "
9	120 "	261 "	141 "
10	126 "	254 "	128 "
11	119 "	238 "	119 "
Total	782 "	1408 "	626 "
Average	111 "	201 "	89 "

GROUP 6

H.C. - Hypophysectomized rats fed ad lib for 28 days.

Animal	Initial Weight	Final Weight	Gain
HC 8	102 gms.	96 gms.	-6 gms.
9	98 "	94 "	-4 "
10	109 "	104 "	-5 "
11	95 "	104 "	+9 "
12	98 "	94 "	-4 "
13	86 "	98 "	+12 "
14	93 "	94 "	+1 "
15	108 "	102 "	-6 "
Total	789 "	786 "	-3 "
Average	98.6"	98.2"	

GROUP 7

P.F.C.H.C. - pair-fed controls for H.C. series for 28 days.

Animal	Initial Weight	Final Weight	Gain
PFCHC 8	106 gms.	114 gms.	+8 gms.
9	96 "	126 "	+30 "
10	97 "	140 "	+43 "
11	95 "	112 "	+17 "
12	85 "	126 "	+41 "
13	85 "	112 "	+27 "
14	90 "	123 "	+33 "
15	106 "	118 "	+12 "
Total	760 "	971 "	211 "
Average	95 "	121 "	26.3"

A detailed report on the results of this investigation will be made at a later date. Further work must be done before the findings can be interpreted. Briefly however, from this study it appears that the periodontal structures have definite advantages for studying osteogenesis. The inhibition of growth by hypophysectomy is clearly apparent in the sections. The same is true of the acceleration of growth by growth hormone. Histologically the growth process appears almost identical to the reparative process described in study 1. If this proves to be true, it permits a study of the factors affecting repair using small animals such as rats, where experimental injury is difficult to produce and measure accurately. In the group of hypophysectomized animals given growth hormone, the bone structure differs from that of the controls. The effect of inadequate caloric intake in group P.T.C.H.C. is apparent histologically. While growth was impaired almost as much as in the hypophysectomized animals, group H.C., a significant difference between the two groups can be clearly seen in the sections. This study also revealed the necessity for cutting sections of the three rat molars in the correct plane. This has proved to be quite difficult to do routinely as the teeth are very small. However, with the co-operation of Mr. Wilson and Dr. Hartroft a method for so doing has been developed and promises to be more satisfactory.

GROUP 7

P.F.C.H.C. - pair-fed controls for H.C. series for 28 days.

Animal	Initial Weight	Final Weight	Gain
PFCHC 8	106 gms.	114 gms.	+8 gms.
9	96 "	126 "	+30 "
10	97 "	140 "	+43 "
11	95 "	112 "	+17 "
12	85 "	126 "	+41 "
13	85 "	112 "	+27 "
14	90 "	123 "	+33 "
15	100 "	118 "	+18 "
Total	760 "	971 "	211 "
Average	95 "	121 "	26.3"

APPENDIX "H"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1953

Subject: Fluoride content of enamel and dentin of teeth from Toronto children studied in relation to Pablum history of the children's diet.

Grantee: Dr. M. Doreen Smith

Project No.: DR 13 Amount of Grant: $500

SUMMARY OF WORK

The results of fluorine analyses on dentin and enamel from a number of teeth in each group have been added to those included in the 1952 report and examined as a whole with respect to the amount of fluorine ingested by the individual either from a water source or from the infant cereal Pablum.

In Section I the teeth are from children, some of whom were recorded as receiving no Pablum during infancy or childhood and others who were reported as having had Pablum at some period in their earlier life. The cereal Pablum was selected since it has been found to contain 6-12 p.p.m. fluorine, a result of its 2% bonemeal content.

The technique for analysis of fluorine and for separation of dentin and enamel has already been reported. The analyses were made by three different technicians.

The results of 46 teeth from Pablum fed and 34 from non-Pablum fed children showed no difference when compared statistically, although the averages of the Pablum fed group was higher for both dentin and enamel. It is likely in some cases that the Pablum was fed for only a short period intermittently. This would weight the results against the Pablum teeth.

In section II the results of the teeth from control area of Sarnia with no fluorine in the water supply are compared with those from Brantford which has artificial fluoridation and those from naturally fluorinated water of Stratford.

The difference in the dentin and enamel of the Stratford teeth is great when compared with the others. The dentin is especially high. The dentin from Brantford teeth is also significantly different from that of the Sarnia teeth when compared by a "t" test at the 1% level. The enamel of the Brantford teeth is significantly different at the 10% level. All averages are shown in a histogram.

Scatter diagrams are presented to show the relationship between age accumulation of fluorine using all teeth analyzed

except those from Brantford. There is some evidence of increased fluorine with age in the dentin of the Stratford teeth which have a wider age range. The enamel does not appear to show this relationship.

The suggestion that fluorine accumulates with age in teeth is not surprising if one considers this has also been found to be the case with the skeletal tissue and that balance experiments on normal human subjects show positive balance with respect to humans.

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March, 1953

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Grantee: M. Doreen Smith

Project No.: DR 13 Amount of Grant: $500

As in the February 1952 report of this project the fluoride composition of dentin and enamel of secondary teeth was examined in relation to the fluoride intake of the individuals from whom the teeth were extracted. A number of teeth have been analyzed and added to each group so that the results are more conclusive.

Section I deals with the teeth from subjects recorded as "Pablum-fed" during infancy which are compared with a control group who received no cereal in the form of Pablum.

Section II gives the results for teeth received from three districts which vary with respect to the fluoride content of the water.

Section I

Widespread interest has been evinced in the addition of fluorides to the water supply because of its effect on the incidence of dental caries. It has generally been accepted that the amount of fluorine contributed to the diet by food is negligible. Nevertheless certain baby foods (Pablum and Pabena) which contain bonemeal have been found to yield 6 - 13 p.p.m. fluorine. These foods are recommended by pediatricians and it has been suggested that the fluorine contributed by bonemeal is in a non-absorbable form.

In the writer's laboratory this problem respecting the absorption and retention of fluorides from a bonemeal-containing cereal has been approached by two different channels. Through fluorine balance studies carried out on human subjects (infants and young adults) it was learned that areasonable proportion of the fluorine from Pablum is absorbed and retained (1).

The second method of obtaining information which might contribute evidence bearing on the absorption and retention of fluoride from Pablum was to carry out a series of analyses on the dentin and enamel

of teeth from children fed Pablum as infants and compare these with results from teeth of children who were not fed this cereal.

This report deals with the results obtained from this second method of studying the problem.

Procedure

Dentin and enamel were separated from 46 teeth of Pablum-fed and 34 teeth of non-Pablum fed children - the age range was from 5 to 17 years. Most of the teeth had the carious section removed through the courtesy of the Faculty of Dentistry at the University of Toronto. The enamel and dentin were separated by the Manley and Hodge technique as previously reported(2). This is a delicate procedure and it was found the fractions had to be examined carefully and sometimes re-centrifuged to assure good separation.

The fluorine analyses were made by the standard chemical methods (3). Teeth were analyzed at random by three different technicians, and on the whole showed similar results when checked.

Results and Discussion

In Tables I and II the results of "non Pablum" and "Pablum" teeth are given. There is only a slight difference in the average fluoride content of the dentin in the "Pablum" and "non Pablum" teeth and when studied statistically it was not significant.

The fluoride content of the enamel of teeth analyzed by the different operators always gave a slightly lower average for the "non Pablum" teeth. When a statistical comparison was made of the difference of all the figures for the enamel of "Pablum" teeth with those of the "non Pablum" (with and without the two irregular results at the end of Table II) the difference is significant only at the 50% level which is negligible.

In the collection of "Pablum" teeth, the cereal was eaten over a period of ten years by two subjects, in the others the period was given as from one to four years. It is likely that in some cases the "Pablum" was given intermittently with other cereals. These would weight results against the "Pablum" teeth.

Section II

Teeth received from Brantford, Stratford and Sarnia were separated into dentin and enamel and analyzed by the same procedure as those in Section I. The three technicians did teeth in each group, and all results were pooled for comparison.

Results and Discussion

The results are shown in Tables III, IV and V. A group of deciduous teeth from Brantford are shown in Table VI and averages of

all results are shown in accompanying histogram.

The highly significant difference in both dentin and enamel of Stratford teeth when compared with Sarnia is apparent. Statistical analyses were carried out on the dentin and the enamel of the Brantford (including results from 9 teeth previously reported (2)) and Sarnia groups. The difference in the dentin was significant at the 1% level and that of the enamel at the 10% level.

Four scatter diagrams (Figures I, II, III and IV) to show the relationship of age of tooth with accumulation of fluorine in the dentin are included. The Stratford group covers a wider range of ages. An examination of these data to see if a linear regression exists between age and fluoride content of the teeth might be worthwhile.

In the fluoride content of the enamel as shown in figures III and IV it can be seen that there is no relationship between age of tooth and content of enamel. This is especially interesting in the Stratford group which is exposed to the fluorine-containing water. Either fluorine is not adsorbed on the enamel surfaces, or if it is it must be again eluted by some constant procedure.

That fluorine accumulate with age in the dentin of teeth is not surprising. Analyses made on foot bones of young and old autopsy cases in our laboratory showed an increase of fluorine with age. Glock, Lowater and Murray (4) in England found a similar range in rib bones. McClure and co-workers found no significant retention of fluorine on balance studies carried out on five young men (5). At higher levels of intake Machle and Largent (6) and Largent and Heyroth(7) found considerable retention of fluorine and in all Miss Ham's balance experiments on three adults there was retention. McClure (8) states that there is a strong probability that a normal increase in the per cent of fluorine in skeletal tissues and perhaps in dental tissues occurs with increasing age.

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TABLE I

FLUORINE IN NON PABLUM TEETH

NO	AGE	SEX	TYPE OF TOOTH	DEGREE OF CARIES	% FLUORINE IN DENTINE	% FLUORINE IN ENAMEL	D/E
1.	4½	F	1st Molar	Moderate	0.0076	0.0024	3.1
2	5	M	1st Molar	Moderate	0.0073	0.0035	2.1
3	6		1st Molar	Moderate	0.0095	0.0024	4.0
4	6		1st Molar	Moderate	0.0078	0.0027	2.9
5	6		1st Molar	Slight	0.0080	0.0030	2.6
6	8		1st Molar	Severe	0.0065	0.0066	1.0
7	9		1st Molar	Severe	0.0088	0.0088	1.0
8	10	F	Bicuspid	None	0.0106	0.0086	1.2
9	10	F	Bicuspid	None	0.0097	0.0055	1.8
10	10	M	Bicuspid	None	0.0061	0.0041	1.5
11	10	M	Bicuspid	None	0.0041	0.0041	1.0
12	12	F	Bicuspid	Moderate	0.0071	0.0036	2.0
13	12	F	Molar	Moderate	0.0076	0.0046	1.7
14	12	F	2nd Molar	Moderate	0.0059	0.0025	2.4
15	12	F	2nd Molar	Severe	0.0069	0.0033	2.1
16	13	F	1st Molar	Severe	0.0102	0.0036	2.9
17	13	F	1st Molar	Severe	0.0120	0.0029	4.0
18	13	F	1st Molar	Severe	0.0152	0.0013	11.0
19	14	F	Bicuspid	Severe	0.0184	0.0026	3.1
20	14	F	Bicuspid	Severe	0.0073	0.0020	3.7
21	14	F	Bicuspid	Slight	0.0097	0.0024	4.0
22	14	F	1st Molar	Severe	0.0075	0.0015	4.9
23	14	F	1st Molar	Severe	0.0107	0.0012	9.3
24	14	F	Bicuspid	Severe	0.0053		
25	14	F	Bicuspid	Severe	0.0061		
26	14	F	2nd Molar	Moderate	0.0073	0.0031	2.3
27	14	F	2nd Molar	Moderate	0.0054	0.0027	2.0
28	15	F	Incisor		0.0133	0.0046	2.9
29	16	F	2nd Molar	Slight	0.0089	0.0027	3.3
30	16	F	3rd Molar	Slight	0.0091	0.0035	2.6
31	16	F	Molar	Moderate	0.0109	0.0015	7.3
32	17	F	Molar	Severe	0.0144	0.0084	1.4
33	17	F	3rd Molar	Moderate	0.0105	0.0046	2.3
34	17	M	Bicuspid	Severe	0.0126	0.0051	2.4
35	9		1st Molar	Moderate	0.0085	0.0265	.3
36	17	M	1st Molar	Severe	0.0112	0.0236	.5
			AVERAGE		0.0091	0.0050	3.0

TABLE II FLUORINE IN PABLUM TEETH

NO.	AGE	SEX	TYPE OF TOOTH	DEGREE OF CARIES	% FLUORINE IN DENTINE	% FLUORINE IN ENAMEL	D/E
L.	5	M	Molar	Moderate	0.0070	0.0046	1.52
2	7		1st Molar	Severe	0,0062	0.0059	1.1
3	7		1st Molar	Severe	0.0080	0.0037	2.1
4	8		1st Molar	Moderate	0.0057	0.0051	1.1
5	8		1st Molar	Severe	0.0064	0.0035	1.8
6	8		1st Molar	Severe	0.0068	0.0068	1.0
7	8		1st Molar	Severe	0.0073	0.0057	1.3
8	10		2nd Molar		0.0066	0.0066	1.0
9	10		1st Molar	Severe	0.0106	0.0060	1.8
10	10	M	1st Molar	Severe	0.0059	0.0031	1.9
11	10	M	1st Molar	Severe	0.0520	0.0749	.7
12	10	M	1st Molar	Severe	0.0062	0.0041	1.5
13	10	F	Bicuspid	Moderate	0.0055	0.0048	1.2
14	10	F	1st Molar	Severe	0.0056	0.0015	3.8
15	10	F	1st Molar	Severe	0.0080	0.0008	10.6
16	10	M	2nd Molar	Moderate	0.0071	0.0031	2.3
17	10	F	2nd Molar	Moderate	0.0051	0.0026	1.9
18	11	F	1st Molar	Severe	0.0049	0.0028	1.7
19	11	M	2nd Molar	Severe	0.0077	0.0045	1.7
20	11	F	1st Molar		0.0088	0.0099	.9
21	11	M	1st Molar	Severe	0.0069	0.0029	2.3
22	11	M	1st Molar	Severe	0.0065	0.0023	2.8
23	12	F	1st Molar		0.0102	0.0126	.8
24	12	M	Bicuspid	None	0.0135		
25	12	M	Bicuspid	None	0.0120	0.0110	1.1
26	12	M	Incisor	None	0.0046	0.0042	1.1
27	12	M	Incisor	None	0.0060	0.0034	1.7
28	12	F	1st Molar	Moderate	0.0083	0.0034	2.4
29	13	M	Bicuspid	Moderate	0.0089	0.0076	1.2
30	13	M	2nd Molar	Moderate	0.0073	0.0024	3.0
31	13	F	Molar	Severe	0.0062	0.0030	2.0
32	13	F	Bicuspid	None	0.0108	0.0060	1.8
33	13	F	Bicuspid	None	0.0080	0.0040	2.0
34	14		Bicuspid		0.0101	0.0051	2.0
35	14	M	1st Molar	Severe	0.0093	0.0037	2.9
36	14	M	1st Molar	Severe	0.0445	0.0147	3.0
37	15	F	Cuspid	None	0.0055	0.0075	.7
38	15	F	Cuspid	None	0.0069	0.0038	2.0
39	15	F	1st Molar	Severe	0.0052	0.0034	1.5
40	15	F	1st Molar		0.0194	0.0113	1.7
41	15	M	1st Molar	Moderate	0.0135	0.0041	3.3
42	17	M	3rd Molar	Moderate	0.0119	0.0058	2.1
43	17	F	3rd Molar	Moderate	0.0069	0.0023	3.0
44	17	F	Molar		0.0085	0.0035	2.4
45		M	2nd Molar	None	0.0088	0.0051	1.7
46		M	Bicuspid	None	0.0118	0.0039	3.0
			AVERAGE		0.0099	0.0066	2.1

TABLE III

FLUORINE CONTENT OF STRATFORD TEETH

NO.	AGE	SEX	TYPE OF TOOTH	DEGREE OF CARIES	% FLUORINE IN DENTINE	% FLUORINE IN ENAMEL	D/E
1.	11	M	Bicuspid	None	0.0215	0.0089	2.4
2	13	M	Upper Molar	Severe	0.0234	0.0114	2.3
3	"	"	Molar	Moderate	0.0283	0.0064	4.4
4	"	"	Upper Molar	Moderate	0.0221	0.0038	5.8
5	14	M	Molar	Moderate	0.0486	0.0145	3.3
6	14	M	Molar	Moderate	0.0361	0.0131	2.7
7	14	F	Incisor	None	0.0306	0.0189	1.6
8	16	F	1st Molar	Severe	0.0317	0.0099	3.2
9	17	M	3rd Molar	None	0.0396	0.0203	1.9
10	17	M	Molar		0.0279	0.0140	1.9
11	17	F	Molar	Moderate	0.0478	0.0200	2.3
12	19	F	Bicuspid	Moderate	0.0317	0.0077	4.1
13	21	M	Molar		0.0467	0.0223	2.1
14	24	M	Wisdom	Slight	0.0556	0.0580	.9
15	24	M	3rd Molar	Severe	0.0579	0.0447	1.2
16	24	F	Molar	None	0.0525	0.0157	3.3
17	24	M	3rd Molar	Severe	0.0723	0.0150	4.8
18	24	F	3rd Molar	None	0.0580	0.0227	2.5
19	25	M	Molar	Moderate	0.0546	0.0354	1.5
20	25	M	Molar	None	0.0600	0.0590	1.0
21	25	F	3rd Molar	Severe	0.0605	0.0062	9.7
22	25	F	Bicuspid	Moderate	0.0788	0.1038	.7
23	25	F	Molar	None	0.0280	0.0069	4.0
24	"	"	Molar	Moderate	0.0303	0.0123	2.4
25	"	"	Molar	None	0.0307	0.0129	2.3
26	"	"	Molar	Moderate	0.0295	0.0108	2.7
27	25	F	Bicuspid	Severe	0.0755	0.0393	1.9
28	26	F	2nd Molar	Moderate	0.0791	0.0282	2.8
29	26	M	Molar	None	0.0335	0.0180	1.8
30	27	F	Molar	Severe	0.0827	0.0353	2.3
31	28	F	Molar	Severe	0.0708	0.0089	7.9
32	28	F	Bicuspid	Moderate	0.0846	0.0125	6.7
33	30	F	Molar	Moderate	0.0684	0.0310	2.2
34	30	M	2nd Molar	Moderate	0.1108	0.0246	4.5
35	"	"	3rd Molar	None	0.1040	0.0338	3.0
36	"	"	Molar	None	0.1221	0.0138	8.8
37	30	F	2nd Molar	Severe	0.0679	0.0143	4.7
38	35	M	Molar	None	0.1040	0.0110	9.4
39	38	M	1st Molar	Moderate	0.0722	0.0147	4.9
40		M	1st Molar	None	0.0560	0.0270	2.0
				AVERAGE	0.0559	0.0222	3.5

Ditto marks indicate teeth from same patient.

TABLE IV

FLUORINE CONTENT OF SARNIA TEETH

NO.	AGE	SEX	TYPE OF TOOTH	DEGREE OF CARIES	% FLUORINE IN DENTINE	% FLUORINE IN ENAMEL	D/E
1.	8	F	1st Molar	Moderate	0.0063	0.0073	0.8
2	8	F	1st Molar	Severe	0.0069	0.0070	1.0
3	9	F	1st Molar	Severe	0.0089	0.0067	1.3
4	11	M	1st Molar	Severe	0.0068	0.0039	1.7
5	11		1st Molar	Moderate	0.0073	0.0017	4.30
6	11		1st Molar	Moderate	0.0089	0.0035	2.5
7	11	F	1st Molar	Severe	0.0107	0.0041	2.6
8	12	M	1st Molar	Severe	0.0107		
9	12	F	1st Molar	Severe	0.0078	0.0032	2.4
10	12	M	Bicuspid	None	0.0075	0.0047	1.5
11	12	F	1st Molar	Moderate	0.0138	0.0036	3.8
12	14	F	1st Molar	Moderate	0.0085	0.0044	1.9
13	14	F	Bicuspid	None	0.0066	0.0056	1.1
14	15	F	Molar	Severe	0.0295	0.0178	1.6
15	15	M	1st Molar	Moderate	0.0121	0.0024	5.0
16	16	M	Bicuspid	Severe	0.0081	0.0087	1.0
17	16	M	1st Molar	Severe	0.0130		
18	16	M	Bicuspid	Moderate	0.0080	0.0033	2.4
19	16	M	1st Molar	Severe	0.0108	0.0025	4.3
20	16	M	1st Molar	Severe	0.0145	0.0024	6.0
21	25	M	Cuspid	Moderate	0.0145	0.0031	4.6
22			1st Molar	Severe	0.0090	0.0036	2.5
23			1st Molar	Moderate	0.0094	0.0022	4.2
24			1st Molar	Moderate	0.0098	0.0035	2.8
				Average	0.0104	0.0048	2.7

TABLE V

FLUORINE CONTENT OF BRANTFORD TEETH

NO.	AGE	SEX	TYPE OF TOOTH	DEGREE OF CARIES	% FLUORINE IN DENTINE	% FLUORINE IN ENAMEL	D/E
1	8	F	1st Molar	Severe	0.019(3)	0.017(9)	1.1
2	8	M	2nd Molar	Severe	0.008(6)	0.009(7)	0.9
3	8	F	1st Molar	Severe	0.017(3)	0.005(2)	3.3
4	12	M	1st Molar	Moderate	0.0115	0.0042	2.6
5	12	M	1st Molar	Moderate	0.0181	0.0040	4.4
6	15	M	Molar	Moderate	0.0201	0.0049	4.1
				AVERAGE (including results of 9 teeth already published)	0.0167	0.0084	2.7

Table VI - Fluorine Content of Brantford Teeth - Deciduous

No	Age	Sex	Type of Tooth	Degree of Caries	%Fluorine in Dentine	% Fluorine in Enamel	D/E
1	5		Molar	None	0.0222	0.0202	.9
2	6		Molar	Severe	0.0067	0.0093	1.4
3	6		Molar	Moderate	0.0102	0.0188	1.8
4	6	M	Molar	Severe	0.0096	0.0011	8.7
5	6	F	Molar	Moderate	0.0112	0.0009	28.9
6	7	F	Molar	Severe	0.0074	0.0039	1.9
7	7		Cuspid	None	0.0018	0.0046	2.6
8	7		Molar	Severe	0.0124	0.0181	1.5
9	8	F	Molar	Severe	0.0178	0.0054	3.3
10	8	M	Molar	Moderate	0.0144	0.0024	6.0
11	8	M	Molar	Severe	0.0113	0.0042	2.7
12	9		Molar	Moderate	0.0085	0.0120	1.4
13	10		Molar	Severe	0.0135	0.0096	.7
14	10		Molar	None	0.0154	0.0143	.9
15	12		Molar	Moderate	0.0075	0.0102	1.4
				AVERAGE	0.0126	0.0082	4.3

FIGURE I

TEETH from STRATFORD

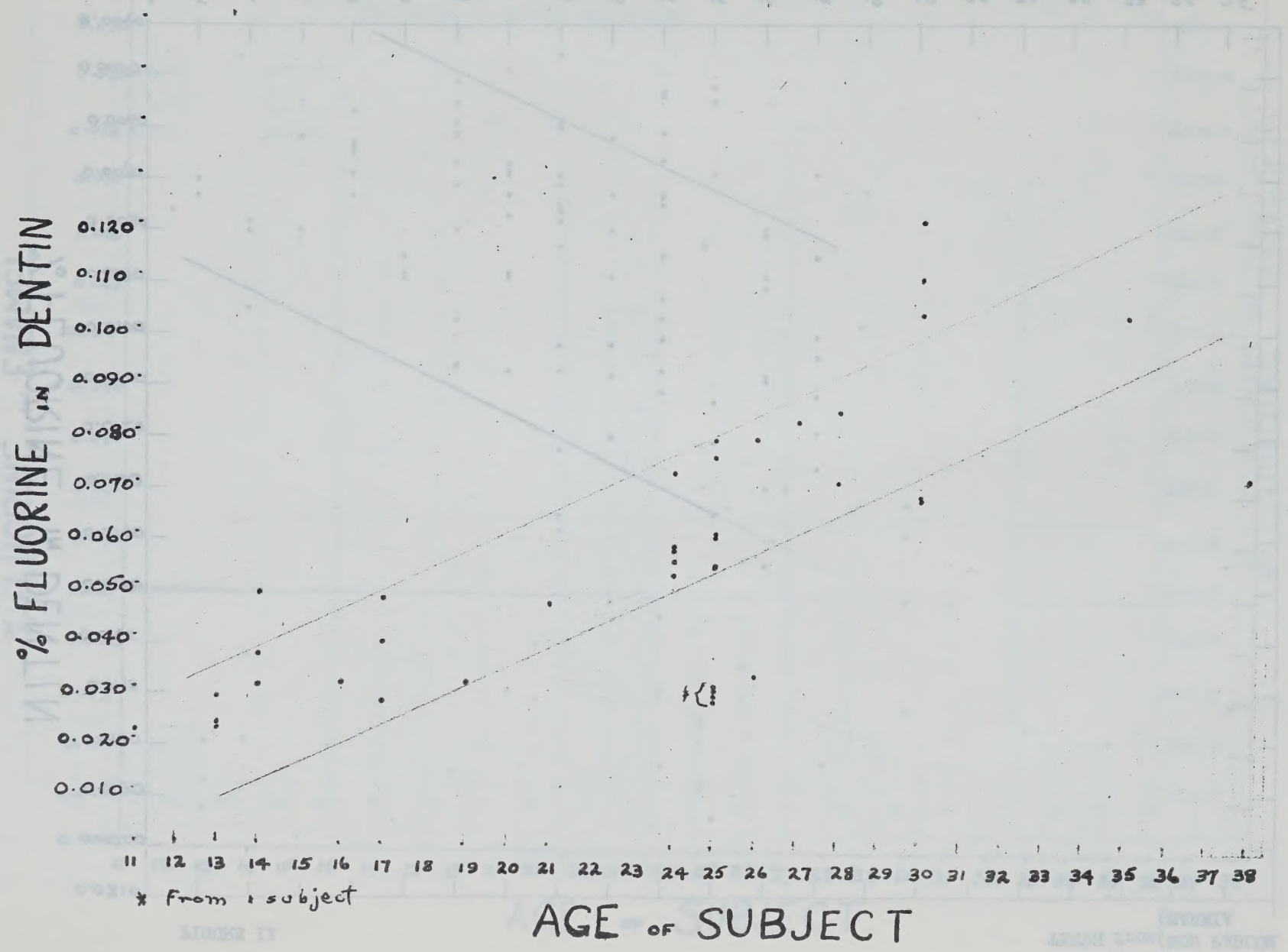

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FIGURE II

TEETH from (PABLUM
(NON PABLUM
(SARNIA

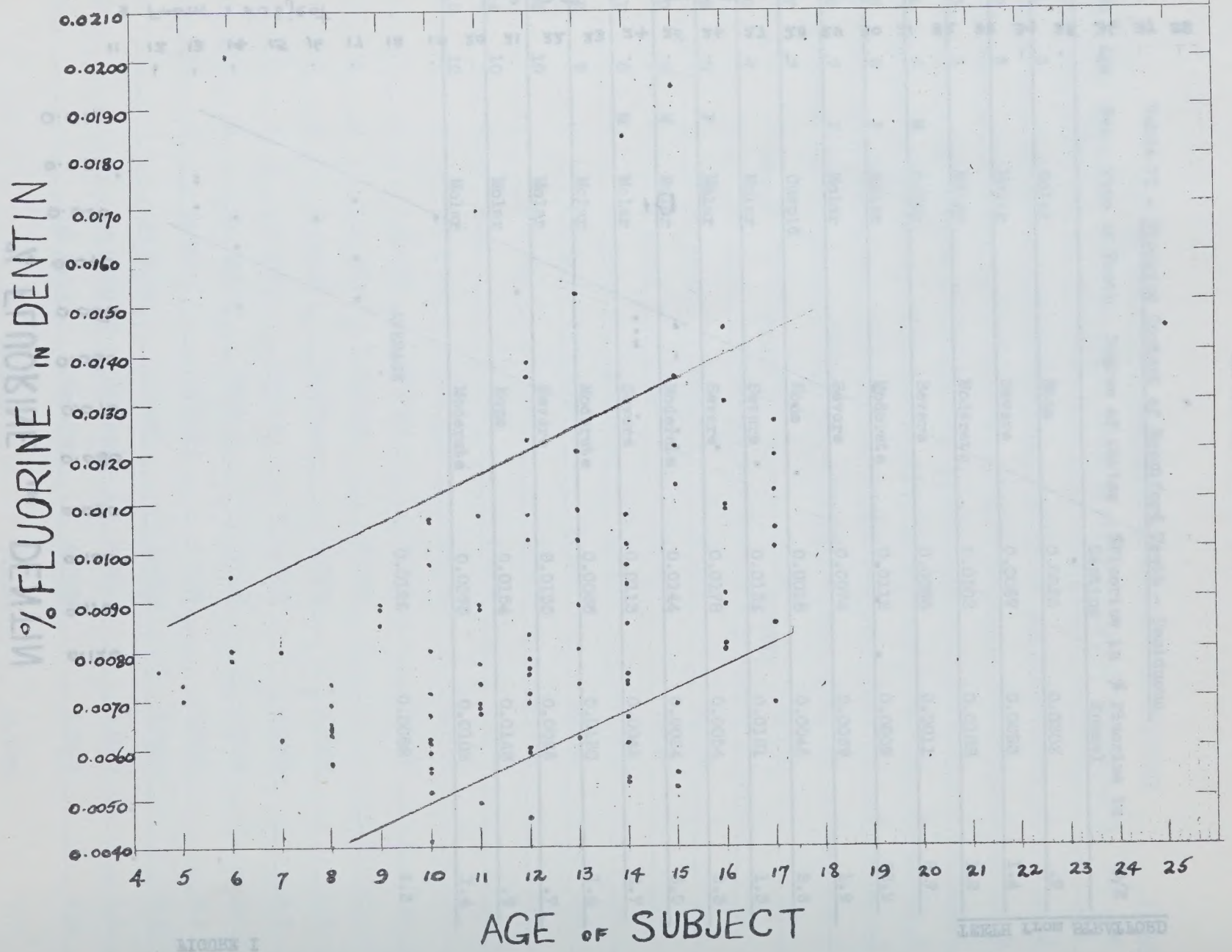


FIGURE III

TEETH from STRATFORD

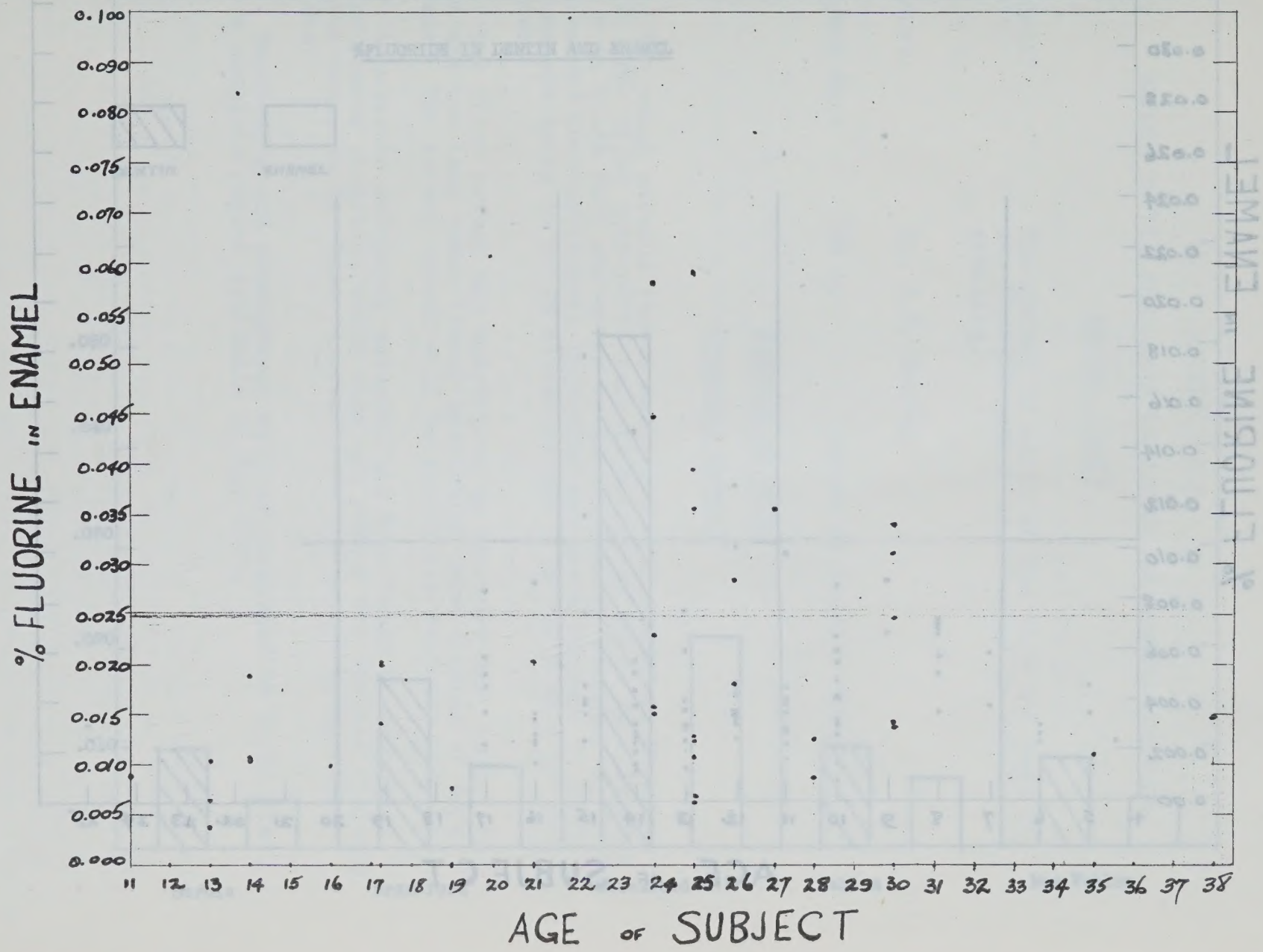
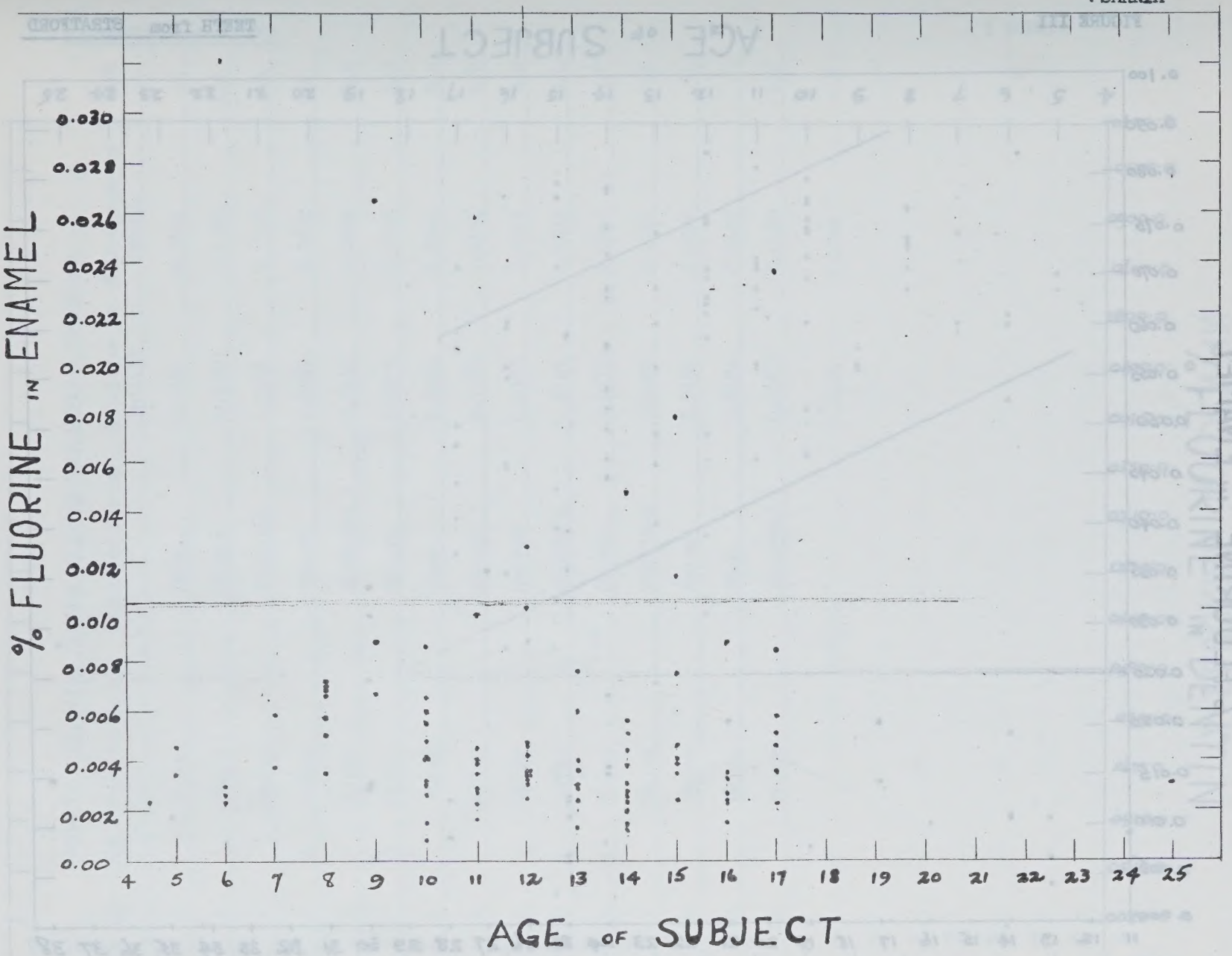
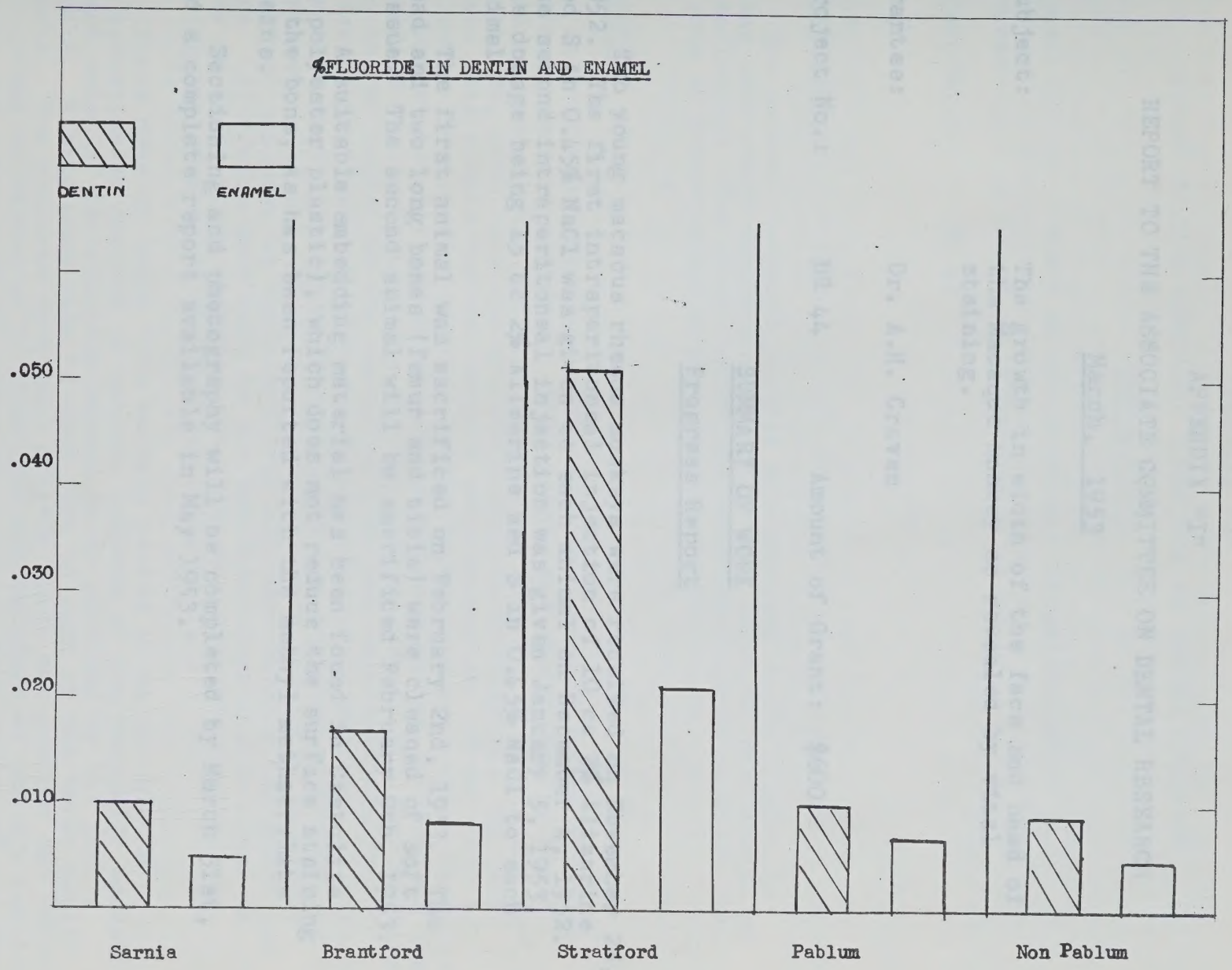


FIGURE IV

TEETH from { PABLUM
{ NON PABLUM
{ SARNIA




%FLUORIDE IN DENTIN AND ENAMEL
DENTIN
ENAMEL
.050
.040
.030
.020
.010
Sarnia
Brantford
Stratford
Pablum
Non Pablum

% FLUORINE IN ENAMEL
AGE
SUBJECT

APPENDIX "I"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

Subject: The growth in width of the face and head of the Macaque monkey as revealed by vital staining.

Grantee: Dr. A.H. Craven

Project No.: DR 44 Amount of Grant: $600

SUMMARY OF WORK

Progress Report

Two young macacus rhesus monkeys were received on November 28, 1952. The first intraperitoneal injection of 10 cc 2% Alizarine Red S in 0.45% NaCl was given to each animal on December 4, 1952. The second intraperitoneal injection was given January 5, 1953, the dosage being 15 cc 2% Alizarine Red S in 0.45% NaCl to each animal.

The first animal was sacrificed on February 2nd, 1953. The head and two long bones (femur and tibia) were cleaned of soft tissue. The second animal will be sacrificed February 9th, 1953.

A suitable embedding material has been found in Castolite (a polyester plastic), which does not reduce the surface staining of the bone, as has been reported with the methyl methacrylate resins.

Sectioning and photography will be completed by March 31st, and a complete report available in May 1953.

APPENDIX "H"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

Subject: The growth in width of the face and head of the Macaque monkey as revealed by vital staining.

Grantee: Dr. A.H. Craven

Project No.: DR 44 Amount of Grant: $600

SUMMARY OF WORK

Progress Report

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A suitable embedding material has been found in Castolite (a polyester plastic), which does not reduce the surface staining of the bone, as has been reported with the methyl methacrylate resins.

Sectioning and photography will be completed by March 31st, and a complete report available in May 1953.

APPENDIX "J"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

Subject: Study of the incidence of malocclusion

Grantee: Dr. R. E. Moyers

Project No.: DR 45

Introduction

The present research project now underway in Burlington is a long term study designed to shed further light on the problem of malocclusion. The chief aim of the investigation is to study the effects of interceptive orthodontic therapy.

Summary

Work of the last four months has involved collecting a sample of three year old children. This sample was restricted to those children born within the year commencing November 1, 1949, and ending October 31, 1950, and who are now living in Burlington. In order to obtain this sample various methods were attempted. These were as follows: (1) a notice was put in the local newspaper which described the project briefly and which advised mothers having children of the specified age to contact the clinic; (2) a list of all the children born in Burlington in the specified year was requested from the office of the Public Health and Welfare doctor in Milton; (3) visits were made to the Hamilton City Hall and the register of births was consulted; (4) all the local physicians were requested to submit a list of children in their practice who were born in the specified year; (5) visits were made to the Hamilton hospitals in order to refer to their birth records. In this case the investigator was once again referred to the Hamilton City Hall.

Since none of these five methods proved to be satisfactory, the investigator decided to contact members of the community herself. A good means of introduction seemed to be provided by the public schools. Visits were made to each form in each of the three public schools. The children were told briefly about the problem and asked if they had relatives or if they knew any children of the age required. In this way a total of 210 names have already been collected and the parents of the majority of these cases have been contacted. Since many of the names suggested were given by very young children a number of them proved to be unsatisfactory, nevertheless, this method proved

to be by far the most successful. Up to the present time a total of 102 mothers have not only consented to have their children take part but many have shown considerable interest in the project. So far, 19 children have actually come to the clinic where they have had their impressions taken and some X-rays made by Doctor Gordon Sayers, the orthodontist working on the project. At the same time a history, compiled by the present investigator, has been filled out with information provided by the mother. (A copy of a history is appended). Appointments are made to coincide as nearly as possible with the child's third birthday.

Future Work

Since it is hoped that a sample of 170 three year old children might be collected, a second round of visits to the public schools is now under way and the high school will also be included.

It is felt that the present project will undoubtedly cause parents, on the whole, in the town to become more aware of the alignment of their children's teeth and consequently they will probably visit their dentists more frequently. For this reason one of the more immediate steps to be followed is the taking of impressions of a sample of six, nine and twelve year old children. These will serve as a control group with which comparisons of the present three year old sample will be made when they reach the proper comparative age.

Although the chief aim of the present project is to study the effects of interceptive orthodontic therapy, nevertheless the possibilities for a number of additional investigations can by no means be overlooked. Work on the eruptive patterns of the deciduous teeth has already been carried out by the present investigator, but it would be particularly advantageous if the present sample of eruptive records could be increased in order to facilitate analyses which have been impossible up to the present time.

It is hoped that one of the first investigations to be completed from the study will be of the incidence of malocclusion in the population. It is important to learn of the prevalence of this defect in a community and so far this problem has not been attempted. It is desirable that within the next six months such a paper will appear.

Conclusion

It is clear that the initial stages in any research program particularly in the field of human investigation are among the most important, especially in the case of a long term study, for the investigator has to insure himself against the loss of too many cases in the years which lie ahead. For this reason children and parents

alike must be carefully handled right from the start in order that they may develop a real interest in the project. Each one of the mothers contacted so far has been personally addressed and any problems which she might have, for example, sucking habits of her children, are discussed as fully as possible. Furthermore, the investigator has offered the use of her car to those mothers who, for various reasons, find it difficult to come to the clinic. Experience has shown that such measures though time consuming help to sustain the interest of the mother and safeguard the future of the whole research project.

NOTE - The application for the continuation of this grant was not made, since we have attempted to bring Dr. Hatton's work work within the budget of the large project. This has been discussed in some detail with Dr. Gordon Brown of the provincial Department of Health, and we have reason to believe that it will be unnecessary for the National Research Council to provide further financial assistance.

PREVENTIVE ORTHODONTICS HISTORY CHART

Pregnancy

No. of pregnancies: No. of children: Miscarriages:

Cause of miscarriage:

Order of pregnancy:

Mother's health during pregnancy: early, middle or late in period of 9 Mos.

Normal: Abnormal:

Diet:

Calcium:

Vitamins:

Additional notes:

PREVENTIVE ORTHODONTICS HISTORY CHART

History of Birth

Mother's age: (at time of birth) Father's age: Order of birth:

Type of Birth: Term: Premature: Late:
Caesarian:

Type of Labour: Normal: Precipitate: Prolonged:
Instruments:
At home: Hospital
Midwife:

Surgery: When:
Drugs:
Anaesthesia:

Presentation: Head: Transverse: Breech: Footling:

Condition after birth: Normal: Blue: Resuscitation:
Convulsions: Jaundice:
Paralysis: Medicine dropper:

Methods of milk feeding: Breast: Bottle: Age of weaning: (from bottle)

Additional notes regarding Milk Diet:

PREVENTIVE ORTHODONTICS HISTORY CHART

Childhood Diseases (date in each case as far as possible)

Measles: Whooping Cough: Vaccine:

Scarlet fever: Chicken pox: Diphtheria:

Diphtheria toxoid: Smallpox: Smallpox vaccination:

Mumps:

Other diseases (what and how severely)

Period of convalescence:

Diet to present time: 1st year:

2nd year:

3rd year:

Notes relative to diet:

Use of teething ring or any sort of comforter:

Age: Time used:

Sucking habits: Thumb: Finger: Which:

How long each day:

Type of sucking:

Notes on sucking:

PREVENTIVE ORTHODONTICS HISTORY CHART

Type of occlusion:

Malocclusion:

In mother: In mother's family:

In father: In father's family:

Subs:

Notes:

Missing teeth (congenital):

In mother: In mother's family:

In father: In father's family:

Subs:

Notes:

History of twinning (multiple births):

Maternal: Paternal: Type of twins:

Nationality of mother: of father:

Education of mother: of father:

Occupation of father:

Consaguinity:

Marked asymmetrics:

APPENDIX "K"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

Subject: An analysis of treated orthodontic cases

Grantee: Dr. R. E. Moyers

Project No.: DR 46 Amount of Grant: $520

SUMMARY OF WORK

This grant has provided funds for the cataloguing and analysis of case histories and cephalograms collected by Dr. G.V. Fisk over a period of years. The filing and cataloguing is not yet complete, but should be finished within six months.

A study is under way at the present time making use of these records. Dr. Matthew Matthews is studying the effectiveness of various appliances in treating similar malocclusions. Dr. Fisk's files include records of cases treated with several mechanisms, including the labio-lingual, twin-wire, pin and tube, and edgewise appliances. This seems to be the first opportunity provided to appraise, cephalometrically, the response to different therapy methods used by the same man.

Results of Dr. Matthews' study and a report of the collection catalogue will be available for the fall meeting of the Committee.

APPENDIX "K"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

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Project No.: DR 46 Amount of Grant: $520

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Results of Dr. Mathews' study and a report of the collection catalogue will be available for the fall meeting of the Committee.

APPENDIX "L"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

Subject: STUDIES IN THE CHEMISTRY OF THE SALIVA. II. THE REDUCING PROPERTY OF SALIVA TOWARDS SOME OXIDATION-REDUCTION INDICATORS.

Grantee: Dr. Gordon Nikiforuk

Project No.: DR 42 Amount of Grant: $1500

SUMMARY OF WORK

The reducing property of saliva towards several oxidation-reduction potential indicators was determined. The indicator dyes selected were those whose E^o values (potentials of half reduced dyes at pH 7.0) covered the range of most biological enzyme systems. It was found that saliva readily reduced dyes whose E^o at pH 7.0 is more positive than -0.29v. This E^o corresponds to that of the lactate-pyruvate system.

The general properties of this phenomenon of the saliva are described. These include (a) effect of pH (b) heat lability (c) effect of some inhibitors (d) effect of some ions (e) rate of reduction of some dyes in different individuals, etc.

The ability of the saliva to reduce redox indicators is proportional to the amount of sediment contained in the saliva. The sediment consists predominantly of bacteria, desquamated epithelium cells and polymorpho-nuclear leukocytes and undetermined solids.

Further work is in progress to determine what portion of the sediment contains the reducing mechanism.

APPENDIX "I"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

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Grantee: Dr. Gordon Nikiforuk

Project No.: DR 42 Amount of Grant: $1500

SUMMARY OF WORK

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The ability of the saliva to reduce redox indicators is proportional to the amount of sediment contained in the saliva. The sediment consists predominantly of bacteria, desquamated epithelium cells and polymorpho-nuclear leukocytes and undetermined solids.

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APPENDIX "L"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

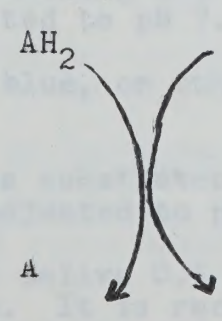
Subject: STUDIES IN THE CHEMISTRY OF THE SALIVA. II. The reducing property of saliva towards several oxidation-reduction indicators.

Grantee: Dr. Gordon Nikiforuk

Project No.: DR 42 Amount of Grant: $1,500

A wide variety of biological processes are intimately linked up with oxidation-reduction reactions. Oxidation and reduction processes may be defined in terms of electron migrations; oxidizing properties result from a tendency to take up electrons, and reducing properties to a tendency to give up electrons. Thus, when one substance is oxidized (parts with electrons) another is simultaneously reduced (takes up electrons). One method for studying quantitatively the oxidation-reduction processes of cells is made possible by the use of oxidation-reduction dye indicators.

Methylene blue and other reducible dyes have been used extensively to study the catalytic transfer of hydrogen (dehydrogenation) by animal and plant tissues and by bacteria (Thunberg, 1920). If methylene blue is added to a suspension of chopped muscle, brain or kidney tissue, or a suspension of bacteria in the presence of a suitable substrate, the dye is rapidly reduced (Quastel and Whethan,1925). The reduction is an indication of the presence of enzymes called dehydrogenases. The reaction with methylene blue may be expressed as follows:



APPENDIX "L"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1951

Subject: STUDIES IN THE CHEMISTRY OF THE SALIVA. II. The reducing property of saliva towards several oxidation-reduction indicators.

Grantee: Dr. Gordon Nikiforuk

Project No.: DR 42 Amount of Grant: $1,500

A wide variety of biological processes are intimately linked up with oxidation-reduction reactions. Oxidation and reduction processes may be defined in terms of electron migrations; oxidizing properties result from a tendency to take up electrons, and reducing properties to a tendency to give up electrons. Thus, when one substance is oxidized (parts with electrons) another is simultaneously reduced (takes up electrons). One method for studying quantitatively the oxidation-reduction processes of cells is made possible by the use of oxidation-reduction dye indicators.

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Oxidizable substrate AH_2 MB (oxidized form - blue)

Dehydrogenase

A MB (reduced form - colorless)

The value of methylene blue is due to the fact that it exists in a colourless reduced form and a coloured (blue) oxidized form.

There is present in whole saliva a number of systems capable of reducing certain oxidation-reduction indicator dyes at varying rates. The purpose of this paper is to report on the general properties of this phenomenon and to determine what fraction of the saliva contains the active components.

METHODS AND MATERIALS

Preliminary experiments were carried out employing standard Thunberg tubes and following the methods established by previous investigators. This method consists of placing the saliva and substrate in the main body of the Thunberg tube which has a side arm to permit evacuation. A hollow stopper in the form of an off-set reservoir, containing the methylene blue is connected to the body of the tube by a standard ground glass joint. The off-set reservoir made it possible to bring together the saliva, substrate and methylene blue after the contents of the tube had been evacuated and warmed to the required temperature. It has been observed by Quastel and Wheatley (1932) that reduction of methylene blue was not a straightforward linear function, hence all tubes were read at a point of 90 per cent decoloration.

After preliminary experiments it was found that the above method was not suitable for quantitative measurements in this laboratory. It was difficult to maintain anaerobic conditions with many of the available Thunberg tubes. More important was the fact that sedimentation of the saliva greatly influenced the rate of reduction of the dye. Because this technique was cumbersome for large scale experiments it was abandoned in favour of the simplified method of Friedemann and Hollander (1942).

In this method, instead of using evacuated tubes, the reagents were mixed with melted agar and the reaction observed in the solidified medium. Thus anaerobic conditions were obtained and sedimentation of the tissues was prevented. A large number of simultaneous determinations are possible by this technique. The reagents used are as follows:

1. 2 per cent agar containing 0.5 per cent dibasic sodium phosphate and adjusted to pH 7.1.

2. 0.0005 M methylene blue, or other indicator dye in distilled water.

3. Solutions of various substrates or inhibitors. pH of all solutions was adjusted to pH 7.1.

4. Paraffin-stimulated saliva 0.5 (or 1.0 ml.) collected between 10 - 11 a.m. It is recognized that standardization of collection of saliva is difficult. There are distinct differences between pooled saliva specimens collected from the same individual on different days.

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The procedure consisted of melting the agar and pipetting 3.0 ml. into test tubes (100 mm. X 10 mm.). When the agar had cooled to 42° C., 0.5 ml. of methylene blue or other dye, 0.5 ml. of saliva and 0.5 ml. of substrate when necessary, were added (all previously warmed to 37° C.). Complete admixture of the components was accomplished by immediate shaking. The tubes were immediately plunged into a freezing mixture of ice and salt for one minute. The tubes were then put into a water bath at 37°C. and the change in colour of the dye was observed. All tubes were read at a point of 90 per cent or complete decolourization as stated. The slight diffusion of oxygen from the air into the agar caused a well-demarcated zone of blue colouration.

Definition of Unit Activity:

The reducing coefficient (R.C.) of saliva with respect to redox dyes was defined as the reciprocal of that quantity of saliva which will reduce in one minute to a point of 90 per cent decolourization 0.5 ml. of methylene blue (or other indicator dye) of 5 X 10^{-4} M under standard conditions of temperature and pH.

EXPERIMENTAL FINDINGS

1. Reducing Property of Saliva towards Several Redox Indicators

The reducing property of saliva towards the oxidation-reduction indicators whose E^o values (potentials of half-reduced dyes at pH 7.0) cover the range of most biological enzyme systems was tested. The indicators selected were toluylene blue (E^o = + 0.115v), methylene blue E^o = + 0.011v), 2, 3, 5 triphenyl tetrazolium chloride (E^o = -0.08v), cresyl violet (E^o = -0.170v) and safranin (E^o = -0.289v). The relative position of the various dyes with respect to E^o at a pH 7.0 at 30° C. is illustrated in Figure 1. The scale is arranged between the hypothetical oxygen electrode in equilibrium with 1 atmosphere of oxygen and the pure hydrogen under 1 atmosphere of hydrogen, the difference in potential between these being 1.23 volts. The behaviour of one system towards others may be determined from the scale. Thus the reduced form of any indicator will reduce the oxidized form of all indicators more positive to it, while the oxidized form will oxidize the reduced forms of more negative systems.

Table I indicates the time in minutes required for saliva obtained from laboratory personnel to reduce the various dye indicators.

2. Effect of Boiling Saliva on Reducing Coefficient

Boiling a saliva specimen for five minutes destroys its ability to reduce the above-mentioned redox indicators.

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2. Effect of Boiling Saliva on Reducing Coefficient

Boiling a saliva specimen for five minutes destroys its ability to reduce the above-mentioned redox indicators.

3. Separation of Active Fraction from Whole Saliva

When saliva is permitted to stand at room temperature for 30 minutes the activity is largely recovered in the sediment (approximately 60 per cent). The supernatant is largely inactive.

Saliva passed through a Seitz filter loses its ability to reduce redox indicators.

A sample of pooled saliva was centrifuged for 30 minutes at a speed of 3300 r.p.m. The supernatant was decanted and tested for activity. The sediment was resuspended in an equal volume of 0.1 M phosphate buffer, pH 7.1. The activity of the original saliva, the supernatant and the sediment is given in Table II.

4. Variation in Reducing Coefficient of Saliva Obtained from Different Individuals

Paraffin-stimulated saliva was collected from 51 students whose ages ranged from 20 - 30 years. The distribution curve for this sample is shown in Figure 2. The values for the reducing coefficient X 100 ranged from 0.6 to 16.7 units. The average value for the group was 2.8 units.

5. Correlation Between the Amount of Sediment in the Saliva and its Reducing Coefficient

The amount of sediment (epithelial cells, bacteria, leukocytes and unidentified débris) in a sample of saliva was determined by the use of a "Westergren" sedimentation tube. A measured quantity of saliva (1 ml.) was drawn into the tube, which was placed in a suitable stand and the amount of sediment read in the sedimentation tube units at time intervals of 30, 60, 90, and 120 minutes.

A typical time curve for sedimentation of a saliva sample is shown in Figure 3. Figures used to indicate the amount of sediment represent a reading in the flat third (c) phase of the curve, during which no further packing of the sediment occurred. The relationship between total sediment and reducing coefficient of 51 saliva samples is shown in Figure 4.

6. Effect of pH on the Reducing Coefficient of Pooled Saliva

A 2 per cent agar medium was made with 0.125 M solutions of citrate and phosphate buffer. According to the procedure previously described, 0.25 ml. methylene blue (5 X 10^{-4}M) and a 0.20 ml. aliquot of saliva from a pooled specimen was added to the buffered agar media. The effect of pH, using a phosphate buffer, on the reducing coefficient of saliva is shown in Figure 5. This experiment did not cover a broad enough range on the alkaline side to obtain a satisfactory pH graph.

7. Effect of Inhibitors on Reducing Coefficient of Saliva

Furacin (5-nitro-furaldehyde semicarbazone) has recently been shown to inhibit many dehydrogenases, particularly those involved in carbohydrate metabolism (Asnis and Gots, 1951). Furacin, after incubation with saliva for one hour, inhibited reduction of methylene blue (5×10^{-4} M) at pH 7.1 by 50 per cent at a concentration of 4.2×10^{-5} M. A 50 per cent lowering in reducing coefficient was obtained by a 7.3×10^{-6} M. solution of cyanide, and a 1.3×10^{-4}M solution of iodacetate at pH 7.1. No inhibitory effect was observed by 0.001 M concentrations of sodium fluoride, urethane or penicillin (5,000 units).

8. Effect of Anions on the Reducing Coefficient of Saliva

To separate 0.5 ml. aliquots of pooled saliva 0.5 ml of the following substrates were added: 0.001 M and 0.01 M at pH 7.1 lactate, glutamate, succinate, citrate and formate. The reducing activity of the aliquots was measured according to the methods previously described, immediately and one hour after addition of the substrates. The results of this experiment are shown in Table III (A).

Whole saliva contains large amounts of metabolites (Fosdick and Rapp, 1943). The possibility that such metabolites masked any effect that the added anions may have had was tested by centrifuging 20 ml. of pooled, paraffin-stimulated saliva at 3300 r.p.m. for 30 minutes. The supernatant was decanted; the sediment was then suspended in an equal volume of 0.1 M phosphate buffer. This procedure was repeated with the sediment resuspended in one-half the original volume. The effect of the previously mentioned anions on this fraction is recorded in Table III (B).

DISCUSSION

As a preface to the discussion of experimental results something should be said of the limitations of the use of oxidation-reduction potential indicators. In saliva we have the very anthithesis of a pure chemical compound and it would indeed be difficult for a chemist to prepare or even imagine a more heterogeneous mixture than is found in saliva. The investigator has stated by W.M. Clark (1934) 'makes painfully exact measurements upon frightfully impure compounds'. In measuring oxidation-reduction potentials of biological systems there are also difficulties (Hewitt, 1950): (i) in obtaining reproducible results, since no two aliquots of saliva, even from the same sample, are identical, (ii) in knowing what constituents of the system are instrumental in the changes observed, and (iii) in determining the effects of certain components of the system which indirectly by catalytic, poising or other activities exert an influence on the potentials.

However, measurement of oxidation-reduction potential of saliva is an attempt at a study of a dynamic rather than a static state; of a composite whole rather than an isolated factor. Some interesting facts emerge from a study of the redox systems in saliva.

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However, measurement of oxidation-reduction potential of saliva is an attempt at a study of a dynamic rather than a static state; of a composite whole rather than an isolated factor. Some interesting facts emerge from a study of the redox systems in saliva.

Table I indicates that saliva readily reduces the oxidized form of oxidation-reduction indicators whose E^o at pH 7.0 is more positive than -0.20v. The relative speed at which saliva reduces equimolar solutions of the redox indicators is indicated by the following series: Toluylene blue is reduced faster than (>) methylene blue > 2, 3, 5 triphenyl tetrazolium chloride > cresyl violet > safranin. It is interesting to note that the E^o (e.g., -0.20v) below which the redox indicators are not readily reduced corresponds to the lactate-pyruvate system (Figure 1). This system at pH 7.0 will reduce all indicators more positive than -0.20v but none that are more negative.

The active fraction of the saliva towards redox indicators appears to be highly concentrated in the insoluble sediment. After centrifugation at 3300 r.p.m. for 30 minutes over 70 per cent of the activity is recovered in the sediment (Table II).

The loss of activity in saliva which has been passed through a Seitz filter or in the supernatant if saliva is permitted to slowly sediment while standing further suggests that the actice reducing fraction is associated with the presence of solids in the saliva. These include bacteria, desquamated cells, leukocytes and some debris.

Figure 2 indicates that the reducing coefficient (X100) of different salivas taken from 51 persons, 20 - 30 years varies between 0.6 - 16.7 units. The mean value is 2.8 units. It is highly significant that the data do not fit a normal distribution curve. Rather the curve is skewed to the left, suggesting that other factors rather than chance are responsible for the differences in reducing coefficient.

Saliva specimens could be divided on the basis of the amount of sediment into groups with high and low reducing coefficients. That there is a correlation between total sediment in a saliva sample and its reducing coefficient is indicated by the relationship of these two functions as shown in Figure 4. The dispersion is probably indicative of a lack of homogeniety in the sediment. It will be observed from Figure 3 that during sedimentation of saliva three phases (A, B and C) are noted. A peak value (A phase) is reached and then the amount of sediment decreases (B phase) due to packing reaching a relatively constant value (C). The figures for the amount of sediment represent a reading from C phase of the curve.

The reducing activity of saliva towards redox indicators has a broad optimum pH range of from 7 to 8.4. A 50 per cent decrease in reducing coefficient is observed at a pH 5.5 (approximately) and at a pH 9 (approximately).

The effect of incubating enzyme inhibitors with saliva for one hour at different concentrations is reported. A 50 per cent reduction in reducing coefficient is obtained with a 7.3×10^{-6} M cyanide, 1.3×10^{-4} M iodacetate at pH 7.1. Furacin (5-nitro-furaldehyde

semicarbazone), reported to be a specific inhibitor of many dehydrogenases (Asnis et al, 1951) at a concentration of 4.2×10^{-5} M at pH 7.1 decreased the reducing coefficient units of saliva by 50 per cent. Penicillin (5,000 units), sodium fluoride 1×10^{-3} M, urethane 1×10^{-3} M at pH 7.1, after one hour incubation with saliva did not affect the reducing coeffieient.

Table III (A and B) suggests that there is no demonstrable effect on the reducing coefficient of saliva by anions as lactate, glutamate, succinate, citrate or formate in concentrations of 1×10^{-3} M, pH 7.1. Similarly, no demonstrable effect was noted when the above substrates were added to the centrifuged sediment washed twice by isotonic saline solutions. The inability to demonstrate an increase of the reducing coefficient of saliva upon addition of substrates is probably explained on the basis that a sufficient amount of substrate is present, even in the washed sediment, which when metabolized reduces all the methylene blue. Thus, 1 ml. of 5×10^{-4} M methylene blue is approximately equivalent to 0.09 mg. of glucose, assuming 1 ml. glucose donates 2 atoms of hydrogen. Such minute amounts of glucose or other bacterial decomposition products are probably found in whole saliva or the washed sediment of saliva.

Experimental data (Table II, Figure 4) suggest strongly that the reducing activity of saliva is associated with the insoluble fraction of the saliva. This fraction is composed of bacteria, desquamated epithelial cells, leukocytes and debris. The separation of the insoluble fraction of saliva into cellular and bacterial components would permit further isolation of the active component in the saliva. It appears that the reducing coefficient of saliva may be primarily a function of the total bacterial population in the mouth. General oral hygiene conditions determine to a large extent the total number of anaerobic bacteria in the mouth (Appleton, 1951). The reducing coefficient of saliva may therefore be correlated with the state of oral hygiene. These problems are being further investigated.

CONCLUSIONS

1. Saliva reduces redox indicators whose E^{o} at pH 7.0 more positive than -0.20v. The rate at which equimolar solutions of the indicators are reduced is indicated by the following series: Toluylene blue faster than (>) methylene blue > 2, 3, 5 triphenyl tetrazolium chloride > cresyl violet safranin (only slightly reduced).

2. The fraction of the saliva that exhibits reducing properties toward redox indicators is associated with the insoluble sediment.

3. A correlation between the amount of sediment and the reducing coefficient of saliva has been shown.

4. The reducing coefficient (X 100) of saliva in 51 individuals (20 - 30 years) ranges from 0.6 to 16.7 units.

5. The reducing coefficient is decreased 50 per cent at pH 7.0 by 4.2×10^{-5} M furacin; 7.3×10^{-6} M cyanide and 1.3×10^{-4} M iodacetate. Sodium fluoride, penicillin and urethane had no inhibitory effect.

6. No demonstrable effect by adding the following substrates was observed: citrate, lactate, glutamate, succinate and formate. This is probably explained by the fact that saliva contains endogenous substrates in sufficient amounts to reduce the methylene blue.

7. The reducing coefficient of saliva is not sharply influenced by pH. A broad pH optimum exists between 7 and 8.

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TABLE I

The time in minutes required to reduce different dye indicators, 5×10^{-4} M, by saliva of different individuals at 37° C., pH 7.1.

Dye Indicator	Time Required to Reduce Indicator by Saliva											
	S.J.	P.P.	Y.S.	B.W.	B.H.	W.P.	P.C.	J.S.	J.C.	A.C.	D.G.	E.W.
Totuylene Blue	12	23	32	7	12	10	27	16	46	32	19	41
Methylene Blue	14	35	55	11	19	14	46	23	75	58	24	57
Cresyl Violet	15	50	97	16	31	18	55	26	117	81	33	107
2,3,5 triphenyl tetrazolium chloride	light pink colour produced in all samples between 15-60 minutes											
Safranin	very slightly reduced after incubation overnight (10-15 hours)											

TABLE II

A sample of pooled saliva was centrifuged 30 minutes at 3300 r.p.m. The reducing coefficient of the supernatant and the sediment resuspended in an equal volume of 0.1 M phosphate buffer, pH 7.1 is recorded for duplicate samples

Control Pooled Saliva	Supernatant	Sediment	Recovery of Activity in Sediment (per cent)
43×10^{-3}	1×10^{-3}	29×10^{-3}	70
41×10^{-3}	1×10^{-3}	31×10^{-3}	73

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Control Pooled Saliva	Supernatant	Sediment	Recovery of Activity in Sediment (per cent)
4.3×10^{-3}	1×10^{-3}	2.9×10^{-3}	70
4.1×10^{-3}	1×10^{-3}	3.1×10^{-3}	73

TABLE III

A.	Control	Lactate		Glutamate		Succinate		Citrate		Formate	
	No Substrate	.01 M	.001 M	.01 M	.001 M	.01 M	.001 M	.01 M	.001 M	.01 M	.001 M
Reducing coefficient (X 100) after immediate addition of substrate	2.08	1.88	1.75	2.08	2.08	1.81	2.30	2.40	1.80	2.20	2.50
After incubation of saliva and substrate for one hour	2.00	2.20	1.90	1.90	1.75	2.18	1.90	2.00	1.80	1.80	1.90
B. Effect of Anions on Sediment of Saliva washed twice and Resuspended to one-half the original volume in 0.9% NaCl.											
Reducing coefficient (X 100) immediately after addition of anion	1.0	.92	.84	.91	.90	.92	.88	.89	.88	.91	.87

TABLE III

A.	Control No substrate	Lactate .01 M	Lactate .001 M	Glutamate .01 M	Glutamate .001 M	Succinate .01 M	Succinate .001 M	Citrate .01 M	Citrate .001 M	Formate .01 M	Formate .001 M
Reducing capacity (% of control (100) after immediate addition of substrate	2.08	1.88	1.75	2.08	2.08	1.81	2.30	2.40	1.80	2.50	2.50
After activation of substrate for one hour	2.00	1.20	1.90	1.75	1.90	2.18	1.90	2.00	1.60	1.80	1.90

B. Effect of toluene on Sediment of Bolivia weathered two one-half the original volume in 0.9% NaCl.

Reducing capacity (% of control (100) after addition of substrate	1.0	.92	.84	.91	.90	.92	.88	.89	.88	.91	.87

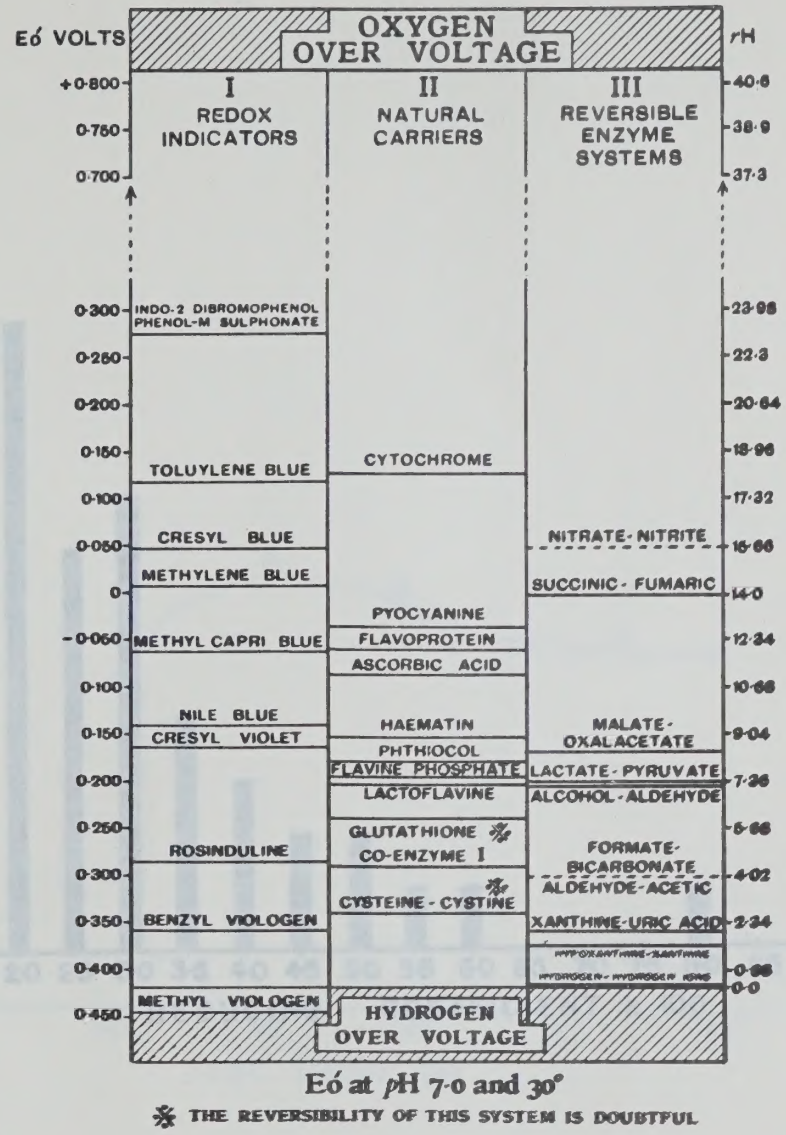

Figure 1. The E° at pH 7.0, 30° C. of some redox indicators and reversible enzyme systems. (From Stephenson, M., Bacterial Metabolism, p. 39)

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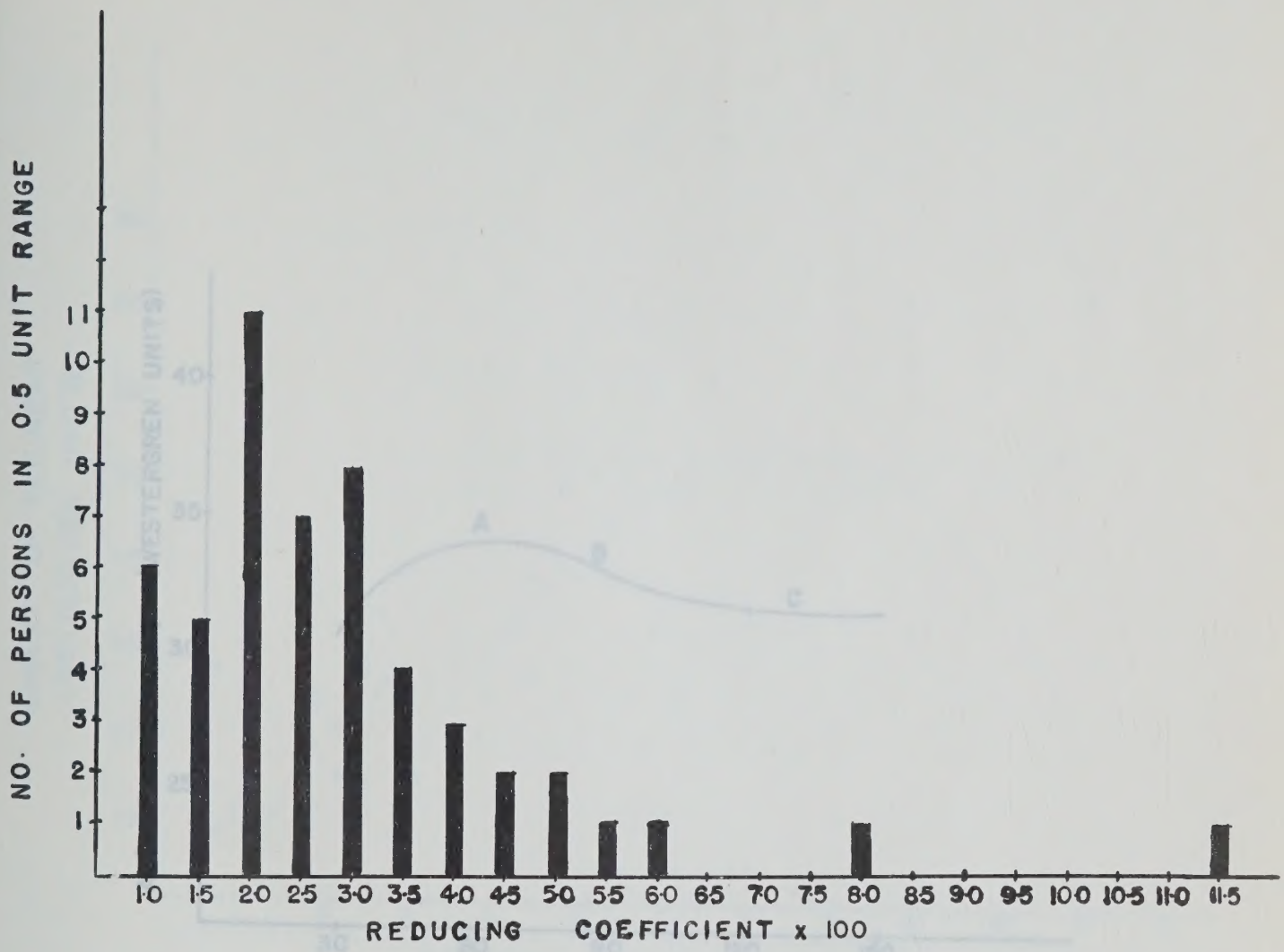

Figure 2. Reducing coefficients of the saliva of 50 different human males, age 20 - 30. Each bar represents the number of individuals in a 0.5 unit.

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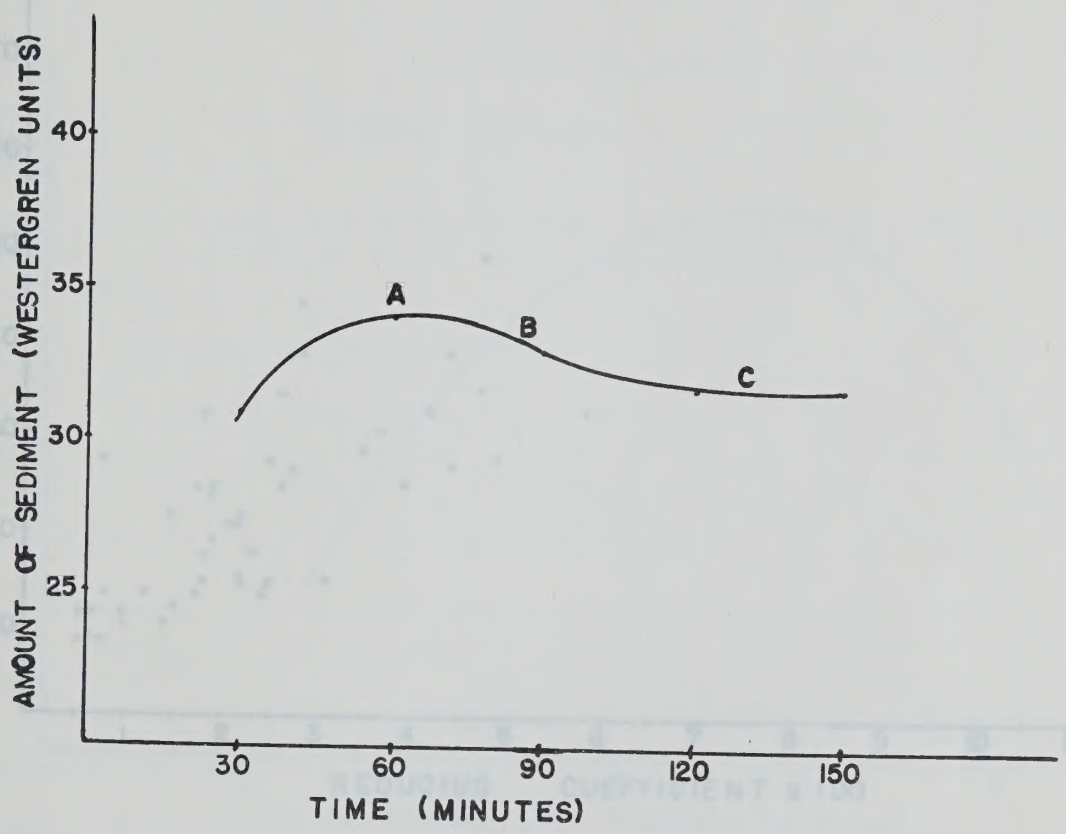

Figure 3. The sedimentation curve of saliva. Sediment is measured by the use of Westergren tubes.

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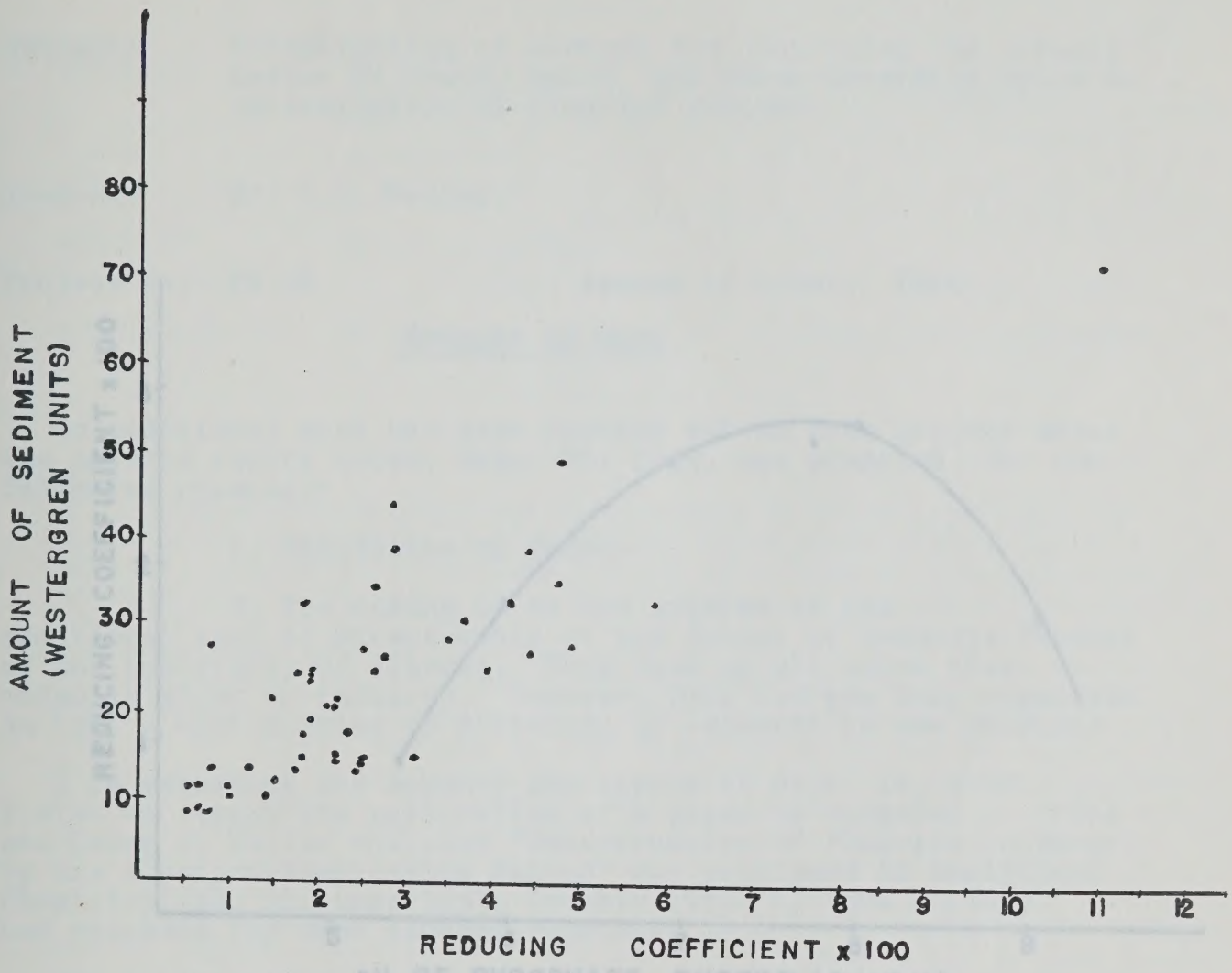

Figure 4. The correlation between the amount of sediment and the reducing coefficients of 50 saliva samples.

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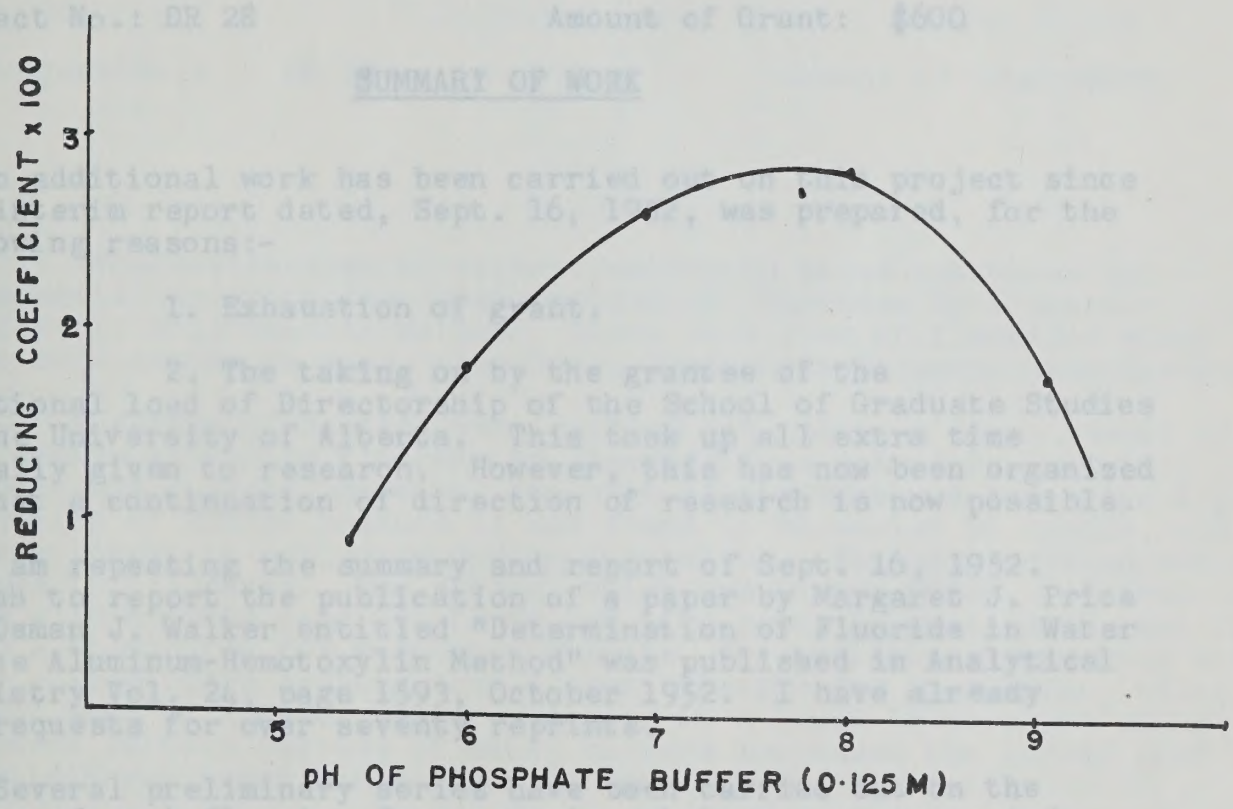

Figure 5. The effect of pH on the reducing coefficient of saliva using phosphate buffer, 0.125 M.

Figure 5. The effect of pH on the reducing coefficient of saliva using phosphate buffer, 0.125 M.

APPENDIX "M"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

Subject: Investigation of methods for destroying the organic matter in teeth, bones, and other materials prior to determination of fluorine content.

Grantee: Dr. O.J. Walker

Project No.: DR 28 Amount of Grant: $600

SUMMARY OF WORK

No additional work has been carried out on this project since the interim report dated, Sept. 16, 1952, was prepared, for the following reasons:-

1. Exhaustion of grant.

2. The taking on by the grantee of the additional load of Directorship of the School of Graduate Studies of the University of Alberta. This took up all extra time normally given to research. However, this has now been organized so that a continuation of direction of research is now possible.

I am repeating the summary and report of Sept. 16, 1952. I wish to report the publication of a paper by Margaret J. Price and Osman J. Walker entitled "Determination of Fluoride in Water by the Aluminum-Hemotoxylin Method" was published in Analytical Chemistry Vol. 24, page 1593, October 1952. I have already had requests for over seventy reprints.

"Several preliminary series have been carried out on the ashing of such fluorine free organic substances as sugar and starch using such alkaline reagents as magnesium nitrate, calcium hydroxide, barium peroxide, and barium hydroxide. The ashing step was satisfactorily completed in about an hour. Additional experiments were carried out in the ashing of the same materials in the presence of the same reagents, and known amounts of fluoride, the products being dissolved in dilute sulfuric acid, distilled, and the fluorine in the distillate determined.

The recovery of fluorine varied in an irregular manner.

Conclusion. More experimental work is necessary in order that a satisfactory procedure be developed."

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"The destruction of organic matter in bones and teeth is essential prior to the determination of fluorides by a photo-electric colorimetric method. There is a loss of fluorides when an acid ashing is used, therefore, basic methods were investigated.

A series of ashings were carried out adding a known amount of fluoride as sodium fluoride to fluoride free materials, namely sugar and starch. However, the bulk of the work was done with 1 gm. samples of starch because it was found to be easier to handle, and less likely to boil over during the ashing. Nickel crucibles were used in all cases. The ashing, which was in two steps, required 15 - 30 minutes over low flame and wire gauze in most cases, but from 30 - 60 minutes for magnesium nitrate, for preliminary charring and 30 - 45 minutes heating over a clay triangle for completion, using full heat of a Meker burner. Ashing at a lower temperature using Bunsen burners instead of Meker burners increased the ashing time but did not improve the fluoride recovery.

To the products of the ashing were added 110 ml. of distilled water and 40 ml. of concentrated sulphuric acid. The mixture was distilled using a 51 cm. condenser with ground glass joints, and the first 90 ml. were collected. No steam escaped and a cool distillate was obtained. Distillation took 15 - 20 minutes.

Several runs were made on teeth using magnesium nitrate. The tooth was dried in an oven for 3 - 4 hours at 110°, and powdered with an agate mortar and pestle. The values varied from 0.016% to 0.040% fluoride. The ashing method however, has not proven to be completely satisfactory, so that these results have little meaning.

The fluoride concentration in the distillate was determined by the sodium alizarin sulphonate photoelectric colorimetric method (1), which is based on the bleaching of the lake formed from a zirconyl salt and sodium alizarin sulphonate by the action of fluoride ion.

A check was run on sulphate carried over in distillation since a concentration of 150 ppm or greater interferes with the results (2). Corrections were applied based on sulphate concentrations found using THQ (tetrahydroxyquinon) indicator.

Magnesium nitrate

One ml. of 20% magnesium nitrate solution was added as ashing agent. Although several runs were carried out, the following are representative of the general results.

mg. F^- added	mg. recovered ↓	% recovered
0.00	0.008	100
0.10	0.104	104
0.15	0.100	67
0.30	0.356	120
0.40	0.400	100

Although some of the results appear to be fairly good there is no explanation for the discrepancies and there is no apparent sequence.

b. Calcium hydroxide

One ml. of 5% calcium hydroxide suspension was added to 1 gm. of starch and known fluoride as ashing agent. The fluoride recovered decreases as the known quantity of fluoride added is increased.

mg. F^- added	mg. recovered	% recovered
0	0.112	100
0.20	0.292	145
0.40	0.400	100
0.45	0.440	98

Blank runs using calcium hydroxide indicate fluoride impurity in the reagent.

c. Barium peroxide

Barium peroxide added in approximately 10% the weight of the organic matter did not retain the fluoride as well as the reagents used previously. Results for barium peroxide + calcium hydroxide were similar to results for calcium hydroxide by itself.

↓ In these tables (M-2 and M-3) the "milligrams recovered" in each case is the actual amount found in the analysis. No allowance has been made in any items for the recovery shown in the blank.

d. Barium hydroxide

Two ml. of 5% barium hydroxide suspension (roughly equal to 1 ml. 5% calcium hydroxide) was used in the same ashing procedure.

The following results show the general trend of the series of runs made.

mg. F^- added	mg. recovered (1)	(2)	Av.	% recovered
0.05	0.12	0.08	0.10	200
0.15	0.13	0.15	0.15	100
0.25	0.26	0.27	0.26	104
0.35	0.24	0.20	0.22	63
0.50	0.29	0.35	0.32	64

The results were high for concentrations from 0.0 to 0.05 mg., and within experimental error from 0.10 to 0.25 mg., but as 0.3 mg. there seems to be a definite break and recovery of the fluoride is poor. Results using larger amounts of barium hydroxide, i.e., 1 gm. barium hydroxide to 1 gm. starch, and results using barium hydroxide + potassium permanganate were similar to those using the barium hydroxide suspension. However, a complete series was not run for these two methods.

e. Potassium hydroxide

Using potassium hydroxide as ashing agent, it appears, from a series of runs that this reagent is the least effective in retaining the fluoride.

Conclusion

The methods of alkaline ashing for destruction of organic matter investigated so far have proven to be unsatisfactory. There seems to be a general tendency to get greater than 100% values for low fluoride concentrations, but poor recovery for higher fluoride concentrations. More experimental work is necessary in order that a satisfactory procedure be developed."

References

1. O.J. Walker and G.C. Gainer, Can. Jour. of Research B 23, 275-280 (1945)
2. O.J. Walker and G.R. Finlay, Can. Jour. of Research B 15, 307 (1937)
3. W.E. Crutchfield, Jr., Ind. Eng. Chem., Anal. Ed. 14, 57-8 (1942)
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References

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APPENDIX "N"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

Subject: The Pathological Characteristics of Experimental Fusospirochetal Infections.

Grantee: H.A. Hunter D.D.S.

Project No. DR 47 Amount of Grant: $1200

SUMMARY OF WORK

The report to date consists of a repetition of the work of Graham. 12 cats, 12 rabbits, and 22 guinea pigs were inoculated subgingivally, intravenously, and subcutaneously respectively with gingival scrapings, guinea pig fusospirochetal exudates and the filtrates of the above.

The local and systemic effects were studied by observation during the experimental period, at autopsy, and by histological sections of the tissues.

The results so far would indicate that only in the cases receiving living organisms were there any significant lesions developed, either locally at the site of inoculation, or systemically in the more remote parts of the body.

Histological examinations will be continued in the immediate future to complete the study of these tissues.

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The presence of "exotoxins" in periodontal lesions has been investigated by Graham (1) who reported that there were highly toxic substances elaborated in periodontal pockets that tended to injure the liver and kidneys of animals. His results were not in accord with those of studies by Rosebury, Clark, Macdonald, and O'Connell (2).

The objectives of this study are the following:-

1. To repeat the work of Graham which has never been confirmed. This is particularly desirable in view of the conflict with the findings of Rosebury et al.

2. To examine the lesions produced by injecting (a) pooled gingival scrapings and, (b) experimental fusospirochetal exudates, and to determine the toxicity of filtrates of such scrapings and exudates.

3. To examine for systemic effects of local experimental fusospirochetal infections.

4. To examine the effects of intravenous injections of the above mentioned exudates and their filtrates.

5. To study the effects of inoculation of pure cultures of component members of the fusospirochetal flora and various recombinations of these organisms.

The influence of the mode and site of entry into the tissues will be tested by using different routes; i.e., subcutaneous, subgingival, and intravenous.

Method

The ultimate source of organisms used in this study was that of human mouths, all of which exhibited clinical symptoms of acute gingivitis or simplex periodontitis. The teeth of these patients were scaled and all the debris removed was made into a heavy suspension

in "DIFCO" heart infusion broth. 1.0 ml. of this suspension was used as a single dose to infect guinea pigs. They were given a subcutaneous injection in the groin with 1.0 ml. of this pooled gingival scrapings suspension. At the end of a week there was nearly always present an acute abscess containing pus in which could be demonstrated a typical fusospirochetal flora. 0.1 ml. of this exudate was injected into another guinea pig in the same manner, and after three or four passages, exudate from several animals was pooled and used for experimental purposes.

To obtain filtrates the above described suspension of pooled gingival scrapings and the pooled guinea pig exudate were each centrifuged for 15 minutes and Seitz-filtered under negative pressure.

Using the resultant filtrates and suspensions, the following experimental procedures were carried out.

Table I.

GROUP I. Inoculated cats subgingivally with:-

(a) 0.5 ml. suspension of pooled gingival scrapings. 4 cats
(b) 0.5 ml. filtrate of pooled gingival scrapings. 4 cats.
(c) 0.5 ml. "DIFCO" heart infusion broth. 4 cats.

Animals sacrificed after 10 days.

GROUP II. Inoculated guinea pigs subcutaneously in the groin with:-

Lot 1. (a) 1.0 ml. suspension of pooled gingival scrapings. 4 G.pigs.
(b) 1.0 ml. filtrate of pooled gingival scrapings. 4 G.pigs.
(c) 1.0 ml. filtrate of pooled guinea pig exudate. 4 G.pigs.

Lot 2. 0.1 ml. pooled guinea pig exudate 4 G.pigs.

Lot 3. 0.075 ml. pooled guinea pig exudate 6 G.pigs.

Animals sacrificed after 7 days if still alive.

GROUP III. Inoculated rabbits intravenously with:-

(a) 0.1 ml. pooled guinea pig exudate 6 rabbits.
(b) 1.0 ml. filtrate of pooled guinea pig exudate 4 rabbits.
(c) No injection - controls 2 rabbits.

Animals to be sacrificed after 7 days.

All animals were autopsied and tissue sections taken of the liver, kidneys, lungs, heart, sternum, pancreas, spleen, adrenals, and the site of the inoculation if a local lesion developed.

The following gross observations were made during the experimental periods of the three groups of animals. In Group I (cats injected subgingivally), two of the four animals in (a) receiving gingival scrapings developed an acute local abscess beneath the left eye, one on the second day, and the other on the sixth day. Both discharged spontaneously, one through the floor of the orbit, and the other through a sinus onto the face just below the rim of the orbit. Both cases healed rapidly after two to four days. Neither animal showed any outward evidence of malaise. The cats in (b) that received inoculations of filtrate of these gingival scrapings and the control animals (c) receiving injections of the heart infusion broth medium did not show any outward changes whatsoever. Two control cats did however refuse to eat, one for the entire experimental period, and the other until the sixth day. They showed a weight loss as a result, but nothing else. This was probably due to unaccustomed confinement.

In Group II (guinea pigs injected subcutaneously), the accompanying Table II summarizes the gross changes recorded in the group of twenty-two guinea pigs inoculated subcutaneously in the groin. In Lot 1 (a) receiving 1.0 ml. of gingival scrapings, it will be seen that all four animals developed acute local abscesses at the site of inoculation. Two of them drained onto the surface and showed signs of healing. Another remained as a non-draining abscess, and the fourth having a phlegmonous extension up the abdominal wall for one inch and containing haemorrhagic areas. The Lot I (b) animals getting 1.0 ml. of filtrate of the above gingival scrapings and Lot I (c) receiving 1.0 ml. of filtrate of guinea pig exudate showed no external effects as a result of their dosage. However, the four animals in Lot 2 having 0.1 ml. of exudate injected into their groins were quickly overwhelmed. One was sacrificed the day following the injection as it was moribund. On the second day two more animals were dead and the fourth so moribund that it was sacrificed. This last animal had no demonstrable lesion prior to its being killed. Lot 3 was a repetition of Lot 2, but with the dose reduced twenty-five per cent. Even so, three of the animals died and the other three were sacrificed by the fourth day. Again, Lot 3 receiving .075 ml. of exudate proved to have a devastating effect upon the treated animals. Three out of six survived the experimental seven day period, though one was developing a case of "snuffles". This is a disease whose causative virus is endemic in rabbits. Of the other three which died, one was overcome on the third day. The second was sacrificed on the fourth day while showing hind quarter paralysis and loss of sense of balance, -- a symptom of coccidiosis. The third rabbit was listless from the first day on and developed snuffles.

The rabbits receiving filtrate injections were, like their counterparts in the cat and guinea pig series, without visible effect from the injections.

Table II.

GROUP II. Guinea pigs inoculated subcutaneously in the groin.

		Amount	Grams Weight Start	Grams Weight End	Inoculum	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Lot 1	(a)	65	300	285	1.0 ml. gingival scrapings	o	o	/	/	/	//	//
		66	325	308		/	/ Dis. Ulc.	/ Dis. Ulc.	/ Dis. Ulc.	/ Dis. Ulc.	/ Dis. Ulc.	/ Dis. Ulc.
		67	325	302		o	o	//	//	//	//	// Dis.
		68	350	294		/	/	/	/	//	//	// P
	(b)	69	325	319	1.0 ml. filtrate scrapings	o	o	o	o	o	o	o
		70	350	349		o	o	o	o	o	o	o
		71	450	411		o	o	o	o	o	o	o
		72	325	328		o	o	o	o	o	o	o
	(c)	77	320	323	1.0 ml. filtrate guin. pig exudate	o	o	o	o	o	o	o
		78	390	387		o	o	o	o	o	o	o
		79	330	326		o	o	o	o	o	o	o
		80	370	367		o	o	o	o	o	o	o
Lot 2		73	400	364	0.1 ml. guin. pig exudate	/	/ died					
		74	325	387		/ Dis. M. Sacr.						
		75	325	265		/ P	M Sacr.					
		76	325	305		o	o died					
Lot 3		81	472	413	0.075 ml. guin. pig exudate	o	/	// P died				
		82	395	351		//	//	/ Ulc. M. Sacr.				
		83	405	262		/	/ Ulc.	/ Ulc.	/ Ulc. Dis. Died			
		84	499	428		o	/	// P died				
		85	322	272		/	//	//	// P M. Sacr.			
		86	332	305		// Dis.	// Dis. Ulc.	// Sacr. Dis. Ulc.				

Legend

o No lesion
/ Palpable mass
// Palpable mass with swelling

P Phlegmon
Dis Discharging abscess
Ulc Necrotic ulceration
M Moribund
Sacr Sacrificed

In Group III (rabbits injected intravenously), the effects are summarized in Table III.

Table III.

Amount	Weight (lbs.) Start	End	Inoculum	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
53	6.25	4.75		o	o	o	o	o	o	o
54	5.75	4.25	0.1 ml. g. pig exudate	M	M	died				
55	5.50	3.75		not eating	not eating	o	o	o	o	o
56	5.00	3.75		o	o	o	paralysis loss balance			
61	5.50	4.75		List.	List.	List.	not eating	o	o	early snuffles
62	5.75	4.75		List.	early snuffles	Wheezing	worse	very sick	died	
57	5.25	5.00		o	o	o	o	o	o	o
58	5.50	5.25	1.0 ml. g. pig filtrate of exudate	o	o	o	o	o	o	o
59	6.00	6.00		o	o	o	o	o	o	o
60	5.00	4.50		o	o	o	o	o	o	o
63	5.25	5.25	None	o	o	o	o	o	o	o
64	6.00	6.00		o	o	o	o	o	o	o

Autopsy Findings

The autopsy findings of the three groups of animals are summarized below.

Cats inoculated subgingivally.

Exudate	#43	Organs grossly normal.
	#45	Organs grossly normal.
	#46	Organs grossly normal.
Filtrate of Scrapings	#47	15 ml. clear ascites.
	#48	20 ml. clear ascites. 3 ml. clear fluid in pericardial sac. Left Kidney: 50% enlarged, pelvis distended with urine. Spleen: enlarged and nodular.
	#49	10 ml. clear ascites. Omentum peppered with pinpoint haemorrhages.
	#50	Spleen: enlarged and nodular. Adrenals: slightly hyperaemic
Controls	#51	Small bowel: hyperaemic. Patches of hard white tissue on parietal wall of abdomen opposite spleen. Also scattered through the omentum.
	#52	Organs grossly normal.
	#41	20 ml. clear ascites.
	#42	Organs grossly normal.

No lesions were demonstrable at the site of injection in the subgingival tissues in the premolar area of the left maxilla.

The presence of both round and flat worms in the small bowel was found in the majority of the cats. These were identified[x] as toxocara mystax and mesocestoides lineatus.

The presence of ascites in all three of the above groups eliminates it as a significant finding so the cause was left undetermined.

Rabbits inoculated intravenously.

Exudate	#53	Liver: pale. Lungs: Sink in water. Whole left and upper lobe of right completely congested. Heart: flabby.
	#54	Lungs: Float, patchy dark red.
	#55	5 ml. clear fluid in pleural cavity, 2 ml. clear fluid in pericardial sac. Left Lung: necrotic. Right Lung: Hyperaemic.
	#62	Lungs: patchy dark red to black. Float in water. No fluid in pleural cavity.

[x] The writer is indebted to Dr. Murray Fallis, Director of the Department of Parasitology, Ontario Research Foundation, who kindly identified these two helminths.

Filtrate of Exudate

#57 20 ml. clear ascites. Lungs: scattered petechial haemorrhages.
#60 20 ml. clear ascites.

Controls

#53 Organs grossly normal.
#64 Organs grossly normal.

Many of the rabbits showed lesions of coccidiosis and two rabbits visibly suffered from snuffles.

Guinea pigs inoculated subcutaneously.

Scrapings

#65 Abscess with thick cheesy pus.
#66 Abscess without gross pus.
#67 Abscess without gross pus.
#68 Abscess with grey smelly pus. Phlegmon to axilla.
Liver: a few 1½ mm. pale yellow spots.

Filtrate of Scrapings

#69 Organs grossly normal.
#70 " " "
#71 " " "
#72 " " "

0.1 ml. Exudate

#73 Organs grossly normal.
#74 " " " 2 mm. necrotic ulcer at injection site, subjacent peritoneum dark red.
#75 Organs grossly normal.
#76 Organs grossly normal.

#77 Organs grossly normal.
#78 Organs grossly normal.
#79 Organs grossly normal.
#80 Organs grossly normal.

#81 Abscess with thin grey pus. Phlegmon up abdominal wall.
#82 Abscess with abundant smelly pus. Phlegmon to axilla.
#83 Abscess with thin grey pus. Phlegmon up abdominal wall.
#84 Abscess with abundant smelly pus. Phlegmon to axilla.
#85 No ulcer. Deep abscess with abundant pus. Phlegmon to axilla.
#86 Abscess, draining.

The results so far would indicate that only in the cases receiving living organisms were there any significant lesions developed, and these developed locally at the site of inoculation, none were seen in the more remote parts of the body.

Histological examinations will be continued in the immediate future to complete the study of these tissues.

References cited

(1) Graham, J.W. The Toxicity of Sterile Filtrates from Parodontal Pockets. Proc. Roy. Soc. Med. (sect. Odont.) 30:1165-1172 Mar. 19, 1937.

(2) Rosebury, T., Clark, A.R., MacDonald, J.B., & O'Connell, D.C. Studies of Fusospirochetal Infection. Part III. Jour. Infect. Dis. 87: 236, Nov.-Dec. 1950.

APPENDIX "O"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

Subject: Demineralization of Hard Tissues by Organic Chelating Agents at Neutral pH's.

Grantee: Dr. Gordon Nikiforuk

Project No. DR 36 Amount of Grant: $1600

SUMMARY OF WORK

The ability of ethylene-diamine tetraacetate to form complexes with calcium salts at neutral and alkaline pH's offers a new and satisfactory method of demineralizing small specimens of hard tissues without subjecting the tissue specimens to low pH's.

This report deals with the histological characteristics of bone tissue demineralized in alkaline solutions of ethylene-diamine tetraacetate under different conditions of concentration, pH, temperature, and fixing solution. The influence of these factors on the staining reactions is compared with similar specimens decalcified by acid solutions.

The results suggest that the chelating agent EDTA is a satisfactory decalcifying agent, suitable for routine histological staining over a wide range of physical conditions.

The histological phase of this project was done by Dr. H.A. Hunter of the Division of Dental Research.

No renewal application is being made for the above grant.

APPENDIX "D"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

MARCH, 1952

Subject: Demineralization of Hard Tissues by Organic Chelating Agents at Neutral pH's.

Grantee: Dr. Gordon Nikiforuk

Project No. DR 36 Amount of Grant: $1500

SUMMARY OF WORK

The ability of ethylene-diamine tetraacetate to form complexes with calcium salts at neutral and alkaline pH's offers a new and satisfactory method of demineralizing small specimens of hard tissues without subjecting the tissue specimens to low pH's.

This report deals with the histological characteristics of bone tissue demineralized in alkaline solutions of ethylene-diamine tetraacetate under different conditions of concentration, pH, temperature, and fixing solution. The influence of these factors on the staining reactions is compared with similar specimens decalcified by acid solutions.

The results suggest that the chelating agent EDTA is a satisfactory decalcifying agent, suitable for routine histological staining over a wide range of physical conditions.

The histological phase of this project was done by Dr. H.A. Hunter of the Division of Dental Research.

Not renewal application is being made for the above grant.

APPENDIX "O"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

Subject: DEMINERALIZATION OF HARD TISSUES BY ORGANIC CHELATING AGENTS AT NEUTRAL pH'S

Grantee: Dr. Gordon Nikiforuk

Project No.: DR 36 Amount of Grant: $1600

Staining Reactions Following Demineralization of Hard Tissues by Chelating and Other Decalcifying Agents

This paper is one of a series dealing with decalcification of hard tissues (bone and teeth) by organic chelating agents at neutral and alkaline pH's.[1] All the usual methods require the use of solutions with low pH's with the exception of the method recommended by Kramer & Shipley[2] using magnesium citrate.

Because of the limited number of techniques available for decalcifying hard tissues at alkaline pH's the present study was planned to observe the effects of using one of the chelating agents - ethylene diamine tetra acetic acid* - a decalcifying medium, and to compare the effects with those of conventional acidulated solutions on the staining reaction of odontal and osseous tissues following the use of haematoxylin and eosin.

It is recognized that the procedures employed in histopathologic techniques are based as much on empiricism as on known scientific methods, and that the differences in colour values and clarity of cytological detail of different sections are necessarily assessed subjectively.

Method

The following experiments were designed to determine the influence on staining reactions of different concentrations, pH's and temperatures of the chelating reagent, different acid decalcifying solutions, and of different fixatives, using rat and guinea pig femurs and mandibles.

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* The ethylene-diamine tetra acetic acid used in this experiment was obtained from the Bersworth Chemical Co., Framingham, Mass., U.S.A., under the trade name "Versene".

The material will be referred to in the text by the abbreviation EDTA. The proper pH's were obtained by neutralizing the acid with sodium hydroxide.

1. "Concentration" Series. Six rat mandibles were decalcified in EDTA solutions at 25°C and at a pH of 7.40 to 7.45, the variable being the concentration of the solution used; i.e., 0.2M to 0.7M, increasing by 0.1 Molar increments.

2. "pH" Series. Four rat femurs were decalcified in EDTA solutions having a constant concentration of 0.25M, but with the pH varied as follows:- (1) 6.02; (2) 7.45; (3) 9.50; (4) 12.01.

3. "Temperature" Series. Four guinea pig femurs were decalcified in 0.5M EDTA solutions at a pH of 7.49. The temperature of the solutions varies as follows:- (1) 4°C; (2) 25°C; (3) 37.5°C; and (4) 60°C.

4. "Decalcifier" Series. Seven guinea pig femurs were decalcified at 25°C in the following solutions:-

(1) 5% nitric acid.
(2) Equal parts 45% formic acid and 20% sodium citrate[4].
(3) 45% formic acid and surplus resin (Amberlite)[x] in the container.
(4) 45% formic acid and surplus resin (Win-3000)[xx] in the container.
(5) Magnesium citrate solution as prepared by Kramer & Shipley[2].
(6) 5% nitric acid with 1% phloroglucin[5].
(7) 0.5M EDTA solution at pH of 7.49.

5. "Fixative" Series. The influence of the fixative medium on the staining reaction observed by using two sets of four guinea pig femurs decalcified identically in EDTA solutions (0.5M at 25°C), but with varying pH's viz. (1) 6.10; (2) 7.49; (3) 9.50; and (4) 11.78. One set was fixed for 48 hours in 10% neutral formalin (pH 7) and the other in Zenker-formol solution for 48 hours. Other sets, fixed as above, were decalcified in 5% nitric acid; 45% formic acid with resin; magnesium citrate; 5% nitric acid with 1% phloroglucin; and 45% formic acid with 20% Na citrate.

All of these tissues were embedded in celloidin (parlodion) and sectioned at 10 microns. Sections were mounted on slides, decelloidinized, and stained together in the same batch of stain (Harris' haematoxylin and eosin Y).

Results

1. "Concentration" Series. The stronger the solution of EDTA, the shorter was the time of decalcification. The time ranged from 8 to 13 days. The staining reactions of all six specimens were indistinguishable. The staining was satisfactory in intensity and clarity.

2. "pH" Series. All four specimens exhibited similar staining reactions and these were well-differentiated and gave good nuclear detail. The

[x] Amberlite IRC-50 (h) manufactured by Rohm & Haas.

[xx] Win-3000 obtained from Winthrop-Stearns Inc.

rate of decalcification was retarded as the pH was increased as follows:- (1) 8 days; (2) 9 days; (3) 16 days; and (4) 18 days. This can be explained on the principle of the solubility product constant.

Bone $\rightleftarrows$ $(Ca)^2 \times (PO_4)^3 = K$

$\downarrow$

EDTA

$\downarrow$

CaEDTA

The higher the pH value the less is the value of the constant.

3. "Temperature" Series. The temperature of the EDTA solutions affected the time taken to decalcify the guinea pig femurs. At 4°C decalcification required 7 days; at 23°C it required 6 days; at 37.5°C, 3 days; and at 60°C it required only 1 day. As a group they stained similarly, but contained too much eosin.

4. "Decalcifier" Series. The time taken by each of the seven solutions to decalcify a guinea pig femur was:-

(1) 5% nitric acid	1 day
(2) 45% formic acid & 20% Na citrate	3 days
(3) " " " & Amberlite	1 day
(4) " " " & Win-3000	1 day
(5) Magnesium citrate, pH 7.5	30 days
(6) 5% nitric acid & 1% phloroglucin	2 days
(7) EDTA (0.5M @ pH 7.49)	6 days

This result clearly showed that the acid solutions were quicker than the alkaline in decalcifying tissues, but that, of the latter, the chelating agent is much faster than the magnesium citrate method.

In the matter of staining reaction, specimens decalcified with nitric acid were not as clear in detail as those decalcified in EDTA, nor did they absorb as much eosin stain. The formic acid - sodium citrate solution produced results that were indistinguishable from those using EDTA, whereas the formic acid and resin specimens were more deeply stained. The clarity using the latter two methods was comparable to that using nitric acid, but poorer than that of EDTA. This may, however, be due to individual tissue variation in this experiment. Tissues decalcified with magnesium citrate gave staining results that had noticeably too much haematoxylin in the nuclei, but the eosin content was quite satisfactory for differentiation and detail. The sections obtained after using nitric acid - with 1% phloroglucin were by far the poorest in nuclear stain. Very little haematoxylin was present in the cell nuclei, though bone matrix took up a fair amount of eosin. The sections were otherwise foggy and the cellular detail was very inadequate.

5. "Fixative" Series. No differences could be detected in the tissues decalcified in EDTA at various pH's and fixed in formalin. The four Zenker-formol fixed tissues also stained uniformly. There was, however, a constant difference between the sets in that the formalin fixed tissues showed more eosin than did the Zenker-formol fixed tissues. Also, the Zenker-formol group showed a denser nuclear stain than did those fixed in formalin. This difference in group staining was observed also in the formic acid-sodium citrate solution. The magnesium citrate decalcified tissues showed the same denser nuclear stain, but similar eosin staining of the sytoplasm, and two of the solutions did not show these differences; i.e., 5% nitric acid and formic acid with resin. Both of these latter two sets stained similarly though too heavily. As mentioned before, the tissues exposed to phloroglucin stained very poorly.

The possible application of EDTA as a decalcifier in studying the distribution of alkaline phosphatase is apparent and is the subject of further studies under progress.

Conclusions

It has been shown that when using the alkaline chelation agent EDTA as a tissue decalcifier the following facts were observed to apply within the experimental limits detailed previously.

(1) The concentration of the solution has no effect on staining.
(2) The temperature of the solution has no effect on staining.
(3) The pH of the solution has no effect on staining.
(4) The staining results obtained were the equal of and in some instances better than when using conventional acid solutions.

This suggests that the chelating agent EDTA is a satisfactory decalcifying medium, suitable for routine histological staining over a wide range of physical conditions.

References cited

1. Nikiforuk, G. & Sreebny, L. Demineralization of hard tissues by organic chelating agents at neutral pH's. Jour. Dent. Res. (in press).

2. Kramer & Shipley, P.G. (in McClung, C.E., Handbook of microscopical technique, New York, Paul B. Hoeber Inc., 1929, p. 260)

3. Dotti, L.B., Paparo, C.P., & Clarke, B.E. The use of ion exchange resin in decalcification of bone. Amer. Jour. Clin. Path. 21: 475, 1951.

4. Morse, A. Formic acid - sodium citrate decalcification and butyl alcohol dehydration of teeth and bones for sectioning in paraffin. Jour. Dent. Res. 24:143, 1945.

5. McClung, C.E. McClung's handbook of microscopical technique. 3rd. ed. New York, Paul B. Hoeber Inc., 1950, p. 273.

APPENDIX "P"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

Subject: Further studies in the electromyography of the head, face and neck.

Grantee: Dr. R.E. Moyers

Project No.: DR 37 Amount of Grant: $800

The work this year has developed in three areas:

A. "An Electromyographic Analysis of Distocclusion" (Dr.R.D.Haryett)

A complete report is not yet available though it is expected to be completed by mid-March. It will be available for the fall meeting of the Committee.

B. "The Physiology of Centric Relation"

This original study has led in two directions, (1) an analysis of the fundamental reflexes governing madibular movements, and (2) study of present misconceptions of clinical problems due to our ignorance of the physiologic mechanism. Of particular interest are age changes in centric relation and changes in both centric relation and physiologic rest position due to orthodontic therapy. Our data continues to demonstrate that neither centric relation nor physiologic rest are as static as once we thought. A final report on this project will be made for the fall meeting of the Committee.

C. "Methods of Recording Centric Relation" (Dr. Charles Moses)

After first studying those factors affecting centric relation, we have analyzed several of the standard methods of recording centric. None is ideal and all introduce sources of possible error. We are attempting to devise a method for recording centric relation which will be as valid and reliable as the electromyographic, and yet applicable to daily practice. A final report on this work will be available for the fall meeting of the Committee.

APPENDIX "D"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

Subject: Further studies in the electromyography of the head, face and neck.

Grantee: Dr. R.E. Moyers

Project No.: DR 37 Amount of Grant: $800

The work this year has developed in three areas:

A. "An Electromyographic Analysis of Dislocclusion" (Dr.R.D.Harvett)

A complete report is not yet available though it is expected to be completed by mid-March. It will be available for the fall meeting of the Committee.

B. "The Physiology of Centric Relation"

This original study has led in two directions, (1) an analysis of the fundamental reflexes governing mandibular movements, and (2) study of present conceptions of clinical problems due to our ignorance of the physiologic mechanism. Of particular interest are age changes in centric relation and changes in both centric relation and physiologic rest position due to orthodontic therapy. Our data continues to demonstrate that neither centric relation nor physiologic rest are as static as once we thought. A final report on this project will be made for the fall meeting of the Committee.

C. "Methods of Recording Centric Relation" (Dr. Charles Moses)

After first studying these factors affecting centric relation, we have analyzed several of the standard methods of recording centric. None is ideal and all introduce sources of possible error. We are attempting to devise a method for recording centric relation which will be as valid and reliable as the electromyograph, and yet applicable to daily practice. A final report on this work will be available for the fall meeting of the Committee.

APPENDIX "Q"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1953

Subject: Electropotentials caused by dental alloys

Grantees: Dr. S.G. Davis and Dr. H.R. MacLean

Grant No.: DR 43 Amount of Grant $1025

SUMMARY

A procedure has been decided for the measurement of the electropotentials produced by dental alloys. Some of the equipment has been ordered. However, more funds are necessary to obtain the complete equipment.

It is proposed to measure the potentials in a saline solution to obtain reproducible results. Attempts will also be made using saliva.

The effects of various cements will be tested in an attempt to reduce the amount of the potential which is produced. It may be possible to obtain an insulating cement.

It is proposed to obtain a graduate in Honors Chemistry as a research assistant for the summer.

APPENDIX "D"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1952

Subject: Electropotentials caused by dental alloys

Grantees: Dr. S.G. Davis and Dr. H.R. Maclean

Grant No.: DR 43 Amount of Grant $1025

SUMMARY

A procedure has been decided for the measurement of the electropotentials produced by dental alloys. Some of the equipment has been ordered. However, more funds are necessary to obtain the complete equipment.

It is proposed to measure the potentials in a saline solution to obtain reproducible results. Attempts will also be made using saliva.

The effects of various cements will be tested in an attempt to reduce the amount of the potential which is produced. It may be possible to obtain an insulating cement.

It is proposed to obtain a graduate in Honors Chemistry as a research assistant for the summer.

APPENDIX "R"

Suggested Research Projects

By decision of the Committee (Min.4(x), 13th Mtg.) projects suggested as research topics are listed hereunder for consideration by Committee members:

	Proposed Subject	Suggested by:	Date
1.	Gold inlays over amalgams Little information is available as to whether gold inlays over amalgams cause disintegration of the cement.	Dr. M.H. Garvin	October, 1950
2.	A study of root canals of teeth This is a very practical research subject as the results would be of value to every practitioner.	Dr. M.H. Garvin	October, 1950
3.	Social aspects of dental disease	Dr. A.C. Burton	September, 1951
4.	The effect of light upon foodstuffs in relation to dentistry.	Dr. M.H. Garvin	March, 1952
5.	To test adhesive qualities of acrylic filling material to tooth structure.	Dr. M.H. Garvin	September, 1952
6.	Determination of the effect of continued dosage of fluorine on periodontal tissues. To make this study it would be necessary to examine the dental records of persons who have lived continuously in an area where fluorine is present in quantity in the water supply.	Dr. M.H. Garvin	March, 1953

APPENDIX "B"

Suggested Research Projects

By decision of the Committee (Min.4(x), 13th Mtg.) projects suggested as research topics are listed hereunder for consideration by Committee members:

Proposed Subject	Suggested by:	Date
1. Gold inlays over amalgams	Dr. M.H. Garvin	October, 1950
Little information is available as to whether gold inlays over amalgams cause disintegration of the cement.		
2. A study of root canals of teeth	Dr. M.H. Garvin	October, 1950
This is a very practical research subject as the results would be of value to every practitioner.		
3. Social aspects of dental diseases	Dr. A.C. Burton	September, 1951
4. The effect of light upon foodstuffs in relation to dentistry.	Dr. M.H. Garvin	March, 1952
5. To test adhesive qualities of acrylic filling material to tooth structure.	Dr. M.H. Garvin	September, 1952
6. Determination of the effect of continued dosage of fluorine on periodontal tissues.	Dr. M.H. Garvin	March, 1953
To make this study it would be necessary to examine the dental records of persons who have lived continuously in an area where fluorine is present in quantity in the water supply.		

APPENDIX "S"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

(i) List of Grantees and Subjects

Grant No.		Grantee	Subject
DR 1	Active	Dr. J.J. Rae, Dept. of Chemistry, University of Toronto	Chemical mechanisms in dental caries
DR 2	Completed	Dr. G.H.W. Lucas, Dept. of Pharmacology, University of Toronto	A study of alveolar oxygen tension and blood oxygen content during nitrous oxide anaesthesia
DR 3	Completed	Dr. H.K. Box, Faculty of Dentistry, University of Toronto	A study of micro-organisms found in deep periodontal pockets and caries lesion as revealed by the electron microscope
DR 4	Completed	Dr. H.K. Box, Faculty of Dentistry, University of Toronto	An investigation of packing material in the treatment of periodontal disease (continuation of investigation previously carried on by Maj. Linghorne)
DR 5	Completed	Major R.L. Twible, Royal Canadian Dental Corps, (later Ontario Research Foundation)	To improve certain properties of the acrylic resin denture base and to effect an economy in denture production
DR 6	Completed	Col. E.M. Wasnbrough, Royal Canadian Dental Corps	To study the effects of altitude acceleration and deceleration on oral tissues
DR 7	Completed	Dr. J.S. Bagnall, Faculty of Dentistry, Dalhousie University	Critical survey of the literature on dental caries
DR 8	Completed	Dr. A.D.A. Mason, Faculty of Dentistry, University of Toronto	A study of the D.M.F. caries rate of the children of Brantford prior to the addition of fluorine to the water supply
DR 9	Completed	Dr. O.J. Walker, Dept. of Chemistry, Dr. H.R. MacLean, Faculty of Dentistry, University of Alberta	An investigation of the effects of certain chemicals on materials in appliances used in prosthetic dentistry

Grant No.		Grantee	Subject
DR 10	Completed	Dr.G.M. Macdonald, Queen Mary Veterans' Hospital, Montreal	Facial Prostheses
DR 11	Completed	Dr. R.J. Godfrey, Faculty of Dentistry, University of Toronto	An anatomical, histological and physiological study of the role of the Condyle in Mastication
DR 12	Completed	Dr. A.E.R. Westman, Ontario Research Foundation	Investigation of cements for methacrylate inlays
DR 13	Completed	Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Fluoride content of enamel and dentin of teeth from Toronto children studied in relation to Pablum history of the children's diets
DR 14	Completed	Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Fluorine analysis of water and food samples from different areas in Ontario collected and studied in relation to incidence of dental caries
DR 15	Completed	Dr. Norma Ford Walker, Dept. of Zoology, University of Toronto	A comparison of the patterns of eruption of the deciduous teeth in man with rates of growth of the dental arches
DR 16	Completed	Dr. C.H. Best, Dept. of Medical Research, University of Toronto	Studies in vitro of certain fungus-like organisms found in periodontal diseases
DR 17	Completed	Dr. R.F. Shaner, Dept. of Anatomy, University of Alberta	An investigation of the effects of solutions used in operative dentistry on the vital pulps of teeth
DR 18	Completed	Dr. H.K. Box, Faculty of Dentistry, University of Toronto	Experimental production of dental caries
DR 19	Completed	Dr. E.A. Sellers, Dept. of Pharmacology, University of Toronto	Tissue toxicity and efficiency of local anaesthetics for topical application and nerve block in dentistry

Grant No.		Grantee	Subject
DR 20	Completed	Dr. Jules Thebaud, Faculty of Dental Surgery, University of Montreal	Improvement of subgingival curettage and surgical techniques used in the treatment of pyorrhea alveolaris
DR 21	Completed	Dr. A.E.R. Westman, Ontario Research Foundation	Investigation of repair techniques for methacrylate dentures
DR 22	Active	Dr. C.H. Best, Dept. of Medical Research, University of Toronto	Part I - An investigation of the mechanism of repair of the supporting structures of the teeth; Part 2 - Fusospirochetal infection and preexisting inflammation
DR 23	Active	Dr. C.P. Leblond, Dept. of Anatomy, McGill University	Entry of phosphate and carbonate salts into teeth as shown with the help of radiophosphorus and radiocarbon
DR 24	Completed	Dr. C.H.M. Williams, Faculty of Dentistry, University of Toronto	The effects of hard and soft diets on the supporting structures of the teeth
DR 25	Completed	Dr. A.E.R. Westman, Ontario Research Foundation	Investigation of methods for the use of methyl methacrylate in direct dental fillings
DR 26	Completed	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	A study of the normal and abnormal physiologic forces of the oral cavity
DR 27	Completed	Dr. J.W. Abbiss, Faculty of Dentistry, Dr. A.G. Nutlay, Faculty of Dentistry, Dalhousie University	A histological study of tooth germ development, both intra and extra alveolar, with a survey of pathological changes in the developing tooth
DR 28	Active	Dr. O.J. Walker, Dept. of Chemistry, University of Alberta	Development of a method for determining fluorine by means of a photo-electric colorimeter

Grant No.		Grantee	Subject
DR 29	Completed	Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Biological absorption of fluorine
DR 30	Completed	Dr. Norma Ford Walker, Dept. of Zoology, University of Toronto	The study of the closure of space following premature loss of deciduous teeth
DR 31	Completed	Dr. L.B. Jacques, Dept. of Physiology, Mr. G.J. Millar, Dept. of Physiology, University of Saskatchewan	An investigation of nerve block
DR 32	Completed	Dr. A.W. Ham, Dept. of Anatomy, University of Toronto	Investigation of the effect of adrenal cortical hormone imbalances on the various connective tissue structures of the growing incisor teeth of animals
DR 33	Completed	Dr. H.A. Hunter, Faculty of Dentistry, University of Toronto	A study of calcifying potential of calcium oleate on the dental pulp
DR 34	Completed	Dr. A.D'Iorio, Dept. of Physiology, University of Montreal	Studies of calcium metabolism in tooth with the aid of Ca^{45}
DR 35	Active	Dr. J.B. MacDonald, Dept. of Bacteriology, University of Toronto	Bacteriology of periodontal disease. -I. Experimental fuso-spirochetal infection with mixtures of pure cultures
DR 36	Completed	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	Demineralization of hard tissues as bone and teeth by organic chelating agents
DR 37	Active	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	Further studies in the electro-myography of the head, face and neck
DR 38	Active	Dr. K.J. Paynter, Faculty of Dentistry, University of Toronto	The effect of thiouracil on the development of molar teeth of rats

rant No.		Grantee	Subject
DR 39	Completed	Dr. A.G. Racey, Faculty of Dentistry, McGill University	Sectioning of teeth and surrounding structures and the effects of potassium deficiency and of pyriodoxine deficiency on the teeth and surrounding structures of dogs
DR 40	Completed	Dr. L.A. Funt, Faculty of Dentistry, University of Toronto	Effect of tooth eruption and function on the growth of the mandible and facial complex on cats
DR 41	Completed	Dr. Harvey Jenkins, Faculty of Dentistry, University of Toronto	A study of the dental arch form and certain of its anatomical relations in a series of clinically excellent dentitions (After Downs (1))
DR 42	Completed	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	Studies in the chemistry of the saliva. II. The reducing property of saliva towards some oxidation-reduction indicators
DR 43	Active	Dr. S.G. Davis, Dept. of Chemistry, Dr. H.R. MacLean, Faculty of Dentistry, University of Alberta	Electro potentials between dental alloys
DR 44	Active	Dr. Arthur Harry Craven, Faculty of Dentistry, McGill University	The growth and width of the face and head of the Macaque monkey as revealed by vital staining
DR 45	Completed	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	Study of the incidence of malocclusion
DR 46	Active	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	An analysis of treated orthodontic cases
DR 47	Active	Dr. H.A. Hunter, Faculty of Dentistry, University of Toronto	The pathological characteristics of experimental fusospirochetal infections

Grant No.		Grantee	Subject
DR 48	Active	Dr. G. Nikiforuk, Assoc. Prof. of Pedodontics, Dept. of Dental Research, University of Toronto, Toronto, Ont.	The effect of topical application of silicones in protecting teeth against acid solutions, in vitro and dental caries produced in vitro
DR 49	Active	Dr. A.H. Craven, Lecturer in Orthodontics, McGill University Dental School, Montreal, Que.	Posture of the head in man
DR 50	Active	Dr. J.B. MacDonald, Chairman, Division of Dental Research, University of Toronto, Toronto, Ont.	The cultivation and characteristics of bacteroides nigrescens
DR 51	Active	Dr. R.L. Saunders, Prof. of Anatomy, Dalhousie University, Halifax, N.S.	Study of human dental pulp blood vessels by X-ray micro-arteriographic methods

APPENDIX "S"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

(ii) Summary of Grants by Year 1945-54

Grant	Grantee	1945-46 $	46-47 $	47-48 $	48-49 $	49-50 $	50-51 $	51-52 $	52-53 $	53-54 $
DR 1	Rae	[illegible]	900	1000 250	1760 150	1000 550	950	960	1530	1680
DR 2	Lucas	1550	2950	1900	1200 400	325	1550	-	-	-
DR 3	Box	1000	500	500	300	100	-	-	-	-
DR 4	Linghorne R.C.D.C.	-	(Box) 2200 1000	2200	-	-	-	-	-	-
DR 5	Twible R.C.D.C.	-	(Ont.Res.-Foundation) 4800		-	-	-	-	-	-
DR 6	Coons R.C.D.C.	-	Wansbrough	-	-	-	-	-	-	-
DR 7	Bagnall	200	300	225	-	75	-	-	-	-
DR 8	Ellis (Mason)	-	1000	completed-		-	-	-	-	-
DR 9	Walker MacLean	-	1000	1000	1100	300	-	-	-	-
DR 10	MacDonald	-	800	completed	-	-	-	-	-	-
DR 11	Godfrey	-	1425 (half-year)	2550	2200	559.07	-	-	-	-
DR 12	Westman	-	-	2550	See DR 21	See DR 25	See DR 25	See DR 25	-	

APPENDIX "S"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

(11) Summary of Grants by Year 1945-54

Grant	Grantee	1945-46 $	46-47 $	47-48 $	48-49 $	49-50 $	50-51 $	51-52 $	52-53 $	53-54 $
DR 1	Rae	900	1000	1760	1000	950	960	1530		1580
DR 2	Lucas	1550	2950	1900	1200	325	1550	-	-	-
DR 3	Box	1000	500	500	300	100	-	-	-	-
DR 4	Lingborne R.C.D.C.	-	(Box) 2200	2200	-	-	-	-	-	-
DR 5	Twible R.C.D.C.	-	(Ont.Res.-Foundation) 4800		-	-	-	-	-	-
DR 6	Coons R.C.D.C.	-	Wansbrough -		-	-	-	-	-	-
DR 7	Bagnall	200	300	2225	-	75	-	-	-	-
DR 8	Ellis (Mason)	-	1000	completed-						-
DR 9	Walker Maclean	-	1000	1000	1100	300	-	-	-	-
DR 10	MacDonald	-	800	completed-			-	-	-	-
DR 11	Godfrey	-	1425	2550	2200	559.07	-	-	-	-
DR 12	Westman	-	-	2550	See DR 21	See DR 25	See DR 25	See DR 45	-	-

(half-year)

Grant	Grantee	1945-46 $	46-47 $	47-48 $	48-49 $	49-50 $	50-51 $	51-52 $	52-53 $	53-54 $
DR 13	Smith	-	-	1050	1455	1475	1475	1600	500	-
DR 14	Smith	-	-	1125	1500	-	-	-	-	-
DR 15	Walker N.F.	-	-	3000	3000	2500	800	-	-	-
DR 16	Best	-	-	1100	1500	-	-	-	-	-
DR 17	Shaner	-	-	1000	-	-	-	-	-	-
DR 18	Box	-	-	1350	1620	1620 458.80	-	-	-	-
DR 19	Sellers	-	-	1300	1575	Ferguson- 350		-	-	-
DR 20	Thébaud	-	-	800	750	750	-	-	-	-
DR 21	Westman	-	-	-	5000	-	-	-	-	-
DR 22	Box (later Best)	-	-	-	2200	2400	3800	2300 875 1350	4200	4200
DR 23	Leblond	-	-	-	2200	2200 400	2400	2400	3000	3000
DR 24	Williams	-	-	-	1420	3080	1735	-	-	-
DR 25	Westman (Ont.Res. Foundation)	-	-	-	-	4000	3000	4000	-	-
DR 26	Moyers	-	-	-	-	1300	150	-	-	-
DR 27	Abbiss Nutlay	-	-	-	-	1000	200	-	-	-

Counties	1971-72 $	1972-73 $	1973-74 $	1974-75 $	1975-76 $	1976-77 $
Humboldt	-	-	-	-	-	-
Inyo	-	-	-	-	-	-
Kern	-	-	-	-	-	-
Kings	-	-	-	-	-	-
Lake	-	-	-	-	-	-
Lassen	-	-	-	-	-	-
Los Angeles	-	-	-	-	-	-
Madera	-	-	-	-	-	-
Marin	-	-	-	-	-	-
Mariposa	-	-	-	-	-	-
Mendocino	-	-	-	-	-	-
Merced	-	-	-	-	-	-
Modoc	-	-	-	-	-	-
Mono	-	-	-	-	-	-
Monterey	-	-	-	-	-	-
Napa	-	-	-	-	-	-
Nevada	-	-	-	-	-	-
Orange	-	-	-	-	-	-
Placer	-	-	-	-	-	-
Plumas	-	-	-	-	-	-
Riverside	-	-	-	-	-	-
Sacramento	-	-	-	-	-	-
San Benito	-	-	-	-	-	-
San Bernardino	-	-	-	-	-	-
San Diego	-	-	-	-	-	-
San Francisco	-	-	-	-	-	-
San Joaquin	-	-	-	-	-	-
San Luis Obispo	-	-	-	-	-	-
San Mateo	-	-	-	-	-	-
Santa Barbara	-	-	-	-	-	-
Santa Clara	-	-	-	-	-	-
Santa Cruz	-	-	-	-	-	-
Shasta	-	-	-	-	-	-
Sierra	-	-	-	-	-	-
Siskiyou	-	-	-	-	-	-
Solano	-	-	-	-	-	-
Sonoma	-	-	-	-	-	-
Stanislaus	-	-	-	-	-	-
Sutter	-	-	-	-	-	-
Tehama	-	-	-	-	-	-
Trinity	-	-	-	-	-	-
Tulare	-	-	-	-	-	-
Tuolumne	-	-	-	-	-	-
Ventura	-	-	-	-	-	-
Yolo	-	-	-	-	-	-
Yuba	-	-	-	-	-	-

Grant	Grantee	1945-46 $	46-47 $	47-48 $	48-49 $	49-50 $	50-51 $	51-52 $	52-53 $	53-54 $
DR 28	Walker O.J. MacLean	-	-	-	-	1000	1000	1000	600	1000
DR 29	Smith	-	-	-	-	1500	375	-	-	-
DR 30	Walker N.F.	-	-	-	-	1200 600	1400 1200	-	-	-
DR 31	Jaques Millar	-	-	-	-	-	1870	188	-	-
DR 32	Ham	-	-	-	-	-	900	-	-	-
DR 33	Hunter	-	-	-	-	-	1500	1600	-	-
DR 34	D'Iorio	-	-	-	-	-	2840	1200 (Lussier)		-
DR 35	MacDonald	-	-	-	-	-	2000	2800	3300	3610
DR 36	Nikiforuk	-	-	-	-	-	-	2400 350	1600	-
DR 37	Moyers	-	-	-	-	-	-	960	800	300
DR 38	Paynter	-	-	-	-	-	-	1850	2610	1000
DR 39	Racey	-	-	-	-	-	-	1600	1600	-
DR 40	Funt Sayers	-	-	-	-	-	-	325	-	-
DR 41	Jenkins	-	-	-	-	-	-	300	-	-
DR 42	Nikiforuk	-	-	-	-	-	-	-	1500	-

Grants	Grantee	1941-42 $	1942-43 $	1943-44 $	1944-45 $	1945-46 $	1946-47 $	1947-48 $	1948-49 $	1949-50 $	1950-51 $	1951-52 $	1952-53 $	1953-54 $
DR 28	Walker O.J. Mrs. Maclean	-	-	-	-	1000	1000	1000	1000	1000	1000	1000	1000	1000
DR 29	Smith	-	-	-	-	1500	575	-	-	-	-	-	-	-
DR 30	Walker N.F.	-	-	-	-	1200	1400	1200	1200	1200	1200	1200	-	-
DR 31	Jagues Miller	-	-	-	-	-	1870	-	-	500	-	-	-	-
DR 32	Hem	-	-	-	-	-	-	-	-	-	-	-	-	-
DR 33	Humber	-	-	-	-	-	1500	1600	1200 (Trustee)	2840	-	-	-	-
DR 34	D'Iorio	-	-	-	-	-	-	-	-	-	-	-	-	-
DR 35	MacDonald	-	-	-	-	-	-	2800	3200	2000	-	-	-	-
DR 36	Nikiforuk	-	-	-	-	-	-	2400	1600	-	-	-	-	-
DR 37	Moyera	-	-	-	-	-	-	960	800	-	-	-	-	-
DR 38	Paynter	-	-	-	-	-	-	1820	2820	-	-	-	-	-
DR 39	Racey	-	-	-	-	-	-	1600	1600	-	-	-	-	-
DR 40	Pumc Sayers	-	-	-	-	-	-	325	-	-	-	-	-	-
DR 41	Jenkins	-	-	-	-	-	-	300	-	-	-	-	-	-
DR 42	Nikiforuk	-	-	-	-	-	-	-	1505	-	-	-	-	-

Grant	Grantee	1945-46 $	46-47 $	47-48 $	48-49 $	49-50 $	50-51 $	51-52 $	52-53 $	53-54 $
DR 43	Davis MacLean	-	-	-	-	-	-	-	150	1025
DR 44	Craven	-	-	-	-	-	-	-	600	1350
DR 45	Moyers	-	-	-	-	-	-	-	1700	-
DR 46	Moyers	-	-	-	-	-	-	-	520	520
DR 47	Hunter	-	-	-	-	-	-	-	1200	2200
DR 48	Nikiforuk	-	-	-	-	-	-	-	-	2240
DR 49	Craven	-	-	-	-	-	-	-	-	400
DR 50	MacDonald	-	-	-	-	-	-	-	-	400
DR 51	Saunders	-	-	-	-	-	-	-	-	1200

Grants	1945-46	46-47	47-48	48-49	49-50	50-51	51-52	52-53
DR 43 Davis	-	-	-	-	-	-	150	1051
Maclean								
DR 44 Craven	-	-	-	-	-	-	600	1350
DR 45 Moyers	-	-	-	-	-	-	1700	-
DR 46 Moyers	-	-	-	-	-	-	520	-
DR 47 Hunter	-	-	-	-	-	-	1200	2200
DR 48 Mikkelsen	-	-	-	-	-	-	-	2240
DR 49 Craven	-	-	-	-	-	-	-	400
DR 50 MacDonald	-	-	-	-	-	-	-	400
DR 51 Saunders	-	-	-	-	-	-	-	1200

APPENDIX "T"

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City of Montreal

Department of Health

IMPORTANCE OF FLUORIDATION AS A PUBLIC HEALTH MEASURE

by

Dr. J.C. Bouillon, D.D.S., M.P.H.,
Chief, Section of Dental Hygiene,
Department of Health of Montreal.

Over a century has passed since Shattuck wrote his famous report on public health in Massachusetts. Since then, great contributions have been made in the field to eliminate or prevent the cause of wide-spread disease on a community basis. Governmental action in the health field has spread to a degree, and has probably proceeded at a faster degree than in other fields, because of the importance of health services, and the fact that private enterprise by itself has been less successful here than in many other fields in meeting the wants of the average man. (1)

Prevention on a community basis has been the foundation of Public Health. Water sanitation and pasteurization have respectively eliminated to all practical purposes, typhoid fever and many milk-borne diseases; smallpox and diphtheria have just about disappeared through public health immunization programs. Iodine programs have practically eliminated goiter. (2) All these public health programs form the foundation on which the progress of public health can be built. One disease however, unique in its prevalence, regardless of race, climate, region or age group, has been a problem in the field of research, and until today, no solution has been offered for its control. This disease is dental caries.

However, through data obtained from several pilot programs, the anticariogenic effect of the fluoridation of communal water supplies substantiates the hypothesis of early investigators, and seems to be well founded; and through this medium, as stated by A.P. Black, "the reduction of the incidence of dental caries gives promise of being one of the landmarks in the history of public health, <u>not only</u> in this generation, but in this <u>century</u>."

If we consider that in 1941, 10% of 2,000,000 who underwent physical examinations for military purposes were rejected because they did not meet the army's dental requirements, we might understand and evaluate more fully the significance of this statement.

Numerous epidemiological studies conducted in widely separated parts of the world clearly demonstrate that the use of fluoride drinking water during the formative period of the teeth is associated with a 60 to 65 per cent reduction in dental caries experience. (3)

It is true that for many years, several procedures regarding the prevention and control of caries have been a basis for research by the dental profession. Dietary and good nutritional habits have been stressed upon, oral hygiene, the reduction in the intake of starches and carbohydrates have been advocated as it was evident that repair work alone would not solve the problem. All these measures, through public health education, have proven to be sound basically. However, due to lack of response from the public at large, the results are not what they should be.

This problem is overcome by the fluoridation of water supplies, as it is a method of mass control that is cheap and simple to administer.

FLUORINE: The Chemical Agent

Fluorine is one of the lightest and most active elements of the "halogen" family. Its discovery is due to the French chemist Henri Moissan by whom it was isolated in 1886. It is the most active element known and combines readily with all other elements, except oxygen and the rare gases. It is a pale yellow gas and is slightly heavier than air. It boils at -- 187°C. and freezes at -- 223°C.

Like chlorine, bromine and iodine, the other three halogens, its compounds are widely distributed in nature. Commercial deposits of calcium fluoride are found in Illinois and Kentucky. Deposits of cryolite, which is a double fluoride of sodium and aluminum, occur in Iceland and Greenland. About 4% of fluorine present as fluoapatite, a double phosphate and fluoride of calcium, are found in the phosphate deposits of Florida, Tennessee and Kentucky. It is present in most soils, in bones and teeth of animals.

Oddly enough sea water contains the optimum concentration at 1 ppm (as a caries inhibitor) and it is possible that someday the Coastal towns may benefit from this natural fluorine content, if a method can be devised to render this source fit for consumption; and it is also found in varying concentrations, in water supplies

throughout the country.

There is one method for desalting sea water, developed during World War II, consisting in the use of a mixture of silver fluo-silicate and lime, but this can be used only for small quantities.

A brochure prepared by the Council on Dental Health of the American Dental Association presents the figures of Hill and co-workers, indicating that out of 6299 public water supplies examined, 1416 or 22.3% contain in excess of 0.6 ppm of Fl. and many of the remaining supplies contain lesser amounts.

In speaking of fluoridation of water supplies, the term "fluoridation" is used rather than "fluorination" because, unlike Chlorine which is added to the water as the element, fluorine is added in the form of one of its compounds. We owe to a chemist H.V. Churchill, the correlation established between the amount of fluorine in water supplies, and the degree of mottled enamel, first noticed throughout the Southern States, later identified at Bauxite, Arkansas, Colorado Springs and Oakley, Idaho. (4)

Although the most widely used compound for fluoridation is sodium fluoride, several other compounds are under consideration for economic reasons. However, sodium fluoride is available in large quantities, is convenient to use and has a high solubility fairly constant over a wide temperature range.

The toxicity and physiological effects of fluorides have been measured through their use in most of the control studies, for establishing fluorine as a public health measure in the inhibition of dental caries. It is a white crystalline salt usually dyed blue or green so as to be distinguished from other chemicals in the treatment plant.

Saturation is obtained at 25°C., and this solution contains approximately 4 per cent by weight of the material which makes it suitable for feeding in solution in small volumetric feeders. Specifically, only 55.6 gallons of water are required to dissolve the 18.8 pounds of 98 per cent sodium fluoride needed to provide a dosage of 1 ppm of fluoride ion to one million gallons of water.

Sodium Silicofluoride is another fluorine compound considered for water fluoridation. It is at present a very promising source of fluorine; and according to McClure, through studies made "in vitro" there should be no difference in its effects as to caries inhibition as compared to sodium-fluoride. The explanation seems quite simple by the fact that sodium silicofluoride added to water with a pH value of 6.0 or above is converted immediately into colloidal silica and sodium fluoride so that in effect the latter chemical is being fed.

Other chemicals used for water fluoridation are Hydrofluosilicic acid and Hydrofluoric acid. Calcium fluoride is also mentioned, but on account of its insolubility, should have no biological effect. Other new compounds will probably be proposed as time passes on, but although availability and the price must be considered, there are other factors which require the greatest consideration, when making the choice of the material to use.

1 - The proposed new fluoridating agent must not be injurious to health, even when ingested daily over a period of years;

2 - The commercially available product must not carrry impurities which might be injurious to health;

3 - Suitable methods must be available to evaluate these impurities if they occur;

4 - It is necessary that the element on being added to water should release the fluorine and convert it into the fluoride ion. So far, the only reliable data bear evidence of the efficacity of sodium fluoride to fulfill all these conditions, although sodium silicofluoride also seems to be just as suitable and much cheaper.

Fluorine, the nutrient

The idea that fluorine may bear some relationship to dental caries is by no means of recent origin: as far back as 1874, Erhardt, a German physician, recommended potassium fluoride tablets for women during gestation and for children during dentition on the assumption that fluorine would protect against dental caries by increasing the hardness and quality of the enamel. (5)

Fluorine is a trace element in nutrition. It is found in practically all foods, although the daily intake through most of them is negligible. The approximate amount of fluorine intake from sources other than water varies from 0.19 to 0.32 m.g.

It is interesting to recall that on June 22nd, 1892, Sir James Crichton Browne addressed the British Dental Association with these works: "The late Dr. George Wilson showed that fluorine is more widely distributed in nature than was before his time supposed, but still, as he pointedout, it is but sparingly present where it does occur and the only channels by which it can apparently find its way into the animal economy are through the siliceous stems of grasses and the outer husks of grain in which it exists in comparative abundance.

"Analysis has proved that the enamel of the teeth contains more fluorine in the form of fluoride of calcium than any other part of the body; and fluorine might indeed be regarded as the characteristic chemical constituent of this structure, the hardest of

all animal tissues, containing 95.5% of salts against 72% in the dentine. As this is so, it is clear that a supply of fluorine, while the development of the teeth is proceeding, is essential to the proper formation of the enamel and that any deficiency in this respect must result in thin and inferior enamel."

And further on, he continued: "I think it well worthy of consideration, whether the reintroduction into our diet, and especially into the diet of child-bearing women and of children, of a supply of fluorine in some suitable natural form -- and what form can be more suitable than that in which it exists in the pellicles of our grain stuffs -- might not do something to fortify the teeth of the next generation." (6)

In 1899, the report of a study by Hempel and Scheffler revealed that non-carious teeth contained more fluorine than carious teeth. (7) Other reports by Tomes on the chemical composition of enamel, Deninger in 1907 advocating a calcium fluoride therapy for the control of Dental caries, indicate that the importance of fluorine intake in dental health was recognized long before today. (8) (9)

(See Table I)

We must not therefore consider fluorine as a dangerous chemical, since it is so widely distributed in our normal food supply. It is indeed a natural constituent of many of our water supplies. It is not a drug and has no therapeutic effect on teeth that are already decayed. It must be thought of only as a prophylactic measure for reducing the incidence of dental decay.

Mottled Enamel

In order to evaluate the full significance of the Endemic dental fluorosis, - dental caries relationship, - it is necessary to consider the first reports dealing with this unusual defect which was to be known later as mottled enamel.

This condition seems to have been recognized by Kuhns as early as 1888, and Eager in 1901, although we owe to Black and McKay the first progressive studies of mottled enamel. These reports were published in 1916, on the result of surveys conducted in Colorado Springs as well as other parts of Colorado, and among Indian tribes in Arizona.

On the basis of his findings, and after describing the condition as a hypocalcification of the enamel, McKay suggested the possibility that the responsible factor could be constituent of the water supply not detectable by the standard water analysis. (10) (11) (12)

TABLE I

This table indicates two groups of foods with their respective fluorine content in ppm a) as consumed and b) as reported in dry substance of food.

FOODS	FLUORINE IN PPM	FOODS	FLUORINE IN PPM
a) FLUORINE REPORTED IN FOOD AS CONSUMED			
Milk	0.07-0.22	Pork chop	- 1.00
Egg white	0.00-0.60	Frankfurters	- 1.70
Egg yolk	0.40-2.00	Round steak	- 1.30
Butter	-1.50	Oysters	- 1.50
Cheese	-1.60	Herring (smoked)	- 3.50
Beef	< -0.20	Canned shrimp	- 4.40
Liver	1.50-1.60	Canned sardines	7.30-12.50
Veal	-0.20	Canned salmon	8.50- 9.00
Mutton	< -0.20	Fresh fish	1.60- 7.00
Chicken	-1.40	Canned mackerel	-26.89
Pork	< -0.20		
b) FLUORINE REPORTED IN DRY SUBSTANCE OF FOOD			
Rice	<-1.00	Honey	- 1.00
Corn	<-1.00	Cocoa	0.50- 2.00
Corn (canned)	<-0.20	Milk chocolate	0.50- 2.00
Oats	-1.30	Chocolate (plain)	- 0.50
Crushed oats	<-0.20	Tea(various brands)	30.00-60.00
Dried beans	-0.20	Cabbage	0.31- 0.50
Whole buckwheat	-1.70	Lettuce	0.60- 0.80
Wheat bran	<-1.00	Spinach	- 1.00
Whole wheat flour	-1.30	Tomatoes	0.60- 0.90
Biscuit flour	-0.00	Turnips	<- 0.20
Flour	1.10-1.20	Carrots	<- 0.20
White bread	-1.00	White potato	<- 0.20
Ginger biscuit	-2.00	Sweet potato	<- 0.20
Rye bread	-5.30	Apples	- 0.80
Gelatin	-0.00	Pineapple(canned)	- 0.00
Dextrose	-0.50	Orange	- 0.22

This hypothesis was substantiated several years later. It was noted that the affection was largely limited to the permanent teeth, and was more severe in certain localities than in others. The teeth affected had a chalky white appearance, and in some instances, showed discrete or confluent pitting taking on a characteristic brown stain, the prevalence increasing with age. (13) It is assumed that the presence of iron salts in the water is the causative agent of this characteristic hue later known as "Colorado brown stain."

Mottled Enamel and Dental Caries

It is a fact that never in the history of public health was a public health measure introduced with such voluminous data and literature, supplying information regarding the effectiveness of the measure. Never have the epidemiological methods been applied in such a brilliant fashion. And it is to the honour of members of the dental profession, if the problem of dental caries seems to be at last on the verge of at least a partial solution.

However, in order to understand and evaluate the full significance of water fluoridation, it is necessary to summarize the epidemiological studies made by F. Dean through which a definite correlation was established between the degree of severity of mottled enamel and the fluorine concentration of drinking water. How fluorine in excessive doses produced one disease, and in small amounts prevented another; if for no other reason, this alone would suffice to immortalize the names of Dean and Al in the field of research, and place them among the list of those who have been benefactors to mankind. Along with Shattuck, Chadwick, Snow, Frost, etc., Dean and his associates will remain among those public health men of whom so little is known, and yet to whom we owe so much.

As started already, these studies, which brought hope towards a possible mass control of dental caries, will probably be recognized as one of the most marvelous pieces of work of the century in the application of the epidemiological methods and established a definite inverse relationship between endemic dental fluorosis and dental caries experience.

However, long before Dean and Al entered the picture, Black and McKay had established the etiologic agent of mottled enamel as being water-borne. In 1906, an enamel defect (14) existing in Colorado Springs was brought to the attention of Black, who was at this time recognized as one of the foremost students of enamel lesions.

Following a complete personal examination, he coined this new pathological condition: mottled enamel. No other similar condition was known to exist throughout the world. Other similarly afflicted communities were surveyed, and it became apparent that one invariable common factor was the continuous use of the domestic water supply

during the years of enamel formation.

It was noted that the waters associated with mottled enamel were derived from almost every conceivable source: shallow wells, clear mountain streams, high mountain lakes. Some of the largest endemic districts used water from drilled wells of varying depths. Other sources of drinking water in highly endemic areas, such as Colorado Springs, were the product of melted snow from the mountain tops.

Three instances, however, verified conclusively the water hypothesis. The first in 1916 was at Britton, S.D., where a change of domestic water supply took place; unknown before the change, mottled enamel became endemic among all the children subsequently born, and who made continuous use of the new water supply which came, in this instance, from a deep well.

The second episode was at Oakley, Idaho, and occurred under similar circumstances. In 1925, mottling of the enamel was confirmed by a thorough examination.

At Bauxite, Arkansas, mottled enamel became endemic throughout the whole community. Several years prior to 1928, a change in the water supply was made from shallow wells to deep wells for sanitary reasons. After the change, severe mottling developed to such an extent that the deep wells were sealed up, and a new source of water was found. Since 1928, the water supply has been obtained from the Saline River.

Several years after the change to the fluorine free water supply, an interesting observation was made. The youngest age group at Bauxite, that is those farthest removed in time from the influence of the old high fluoride water, showed a higher caries rate despite the fact that they had been exposed to risk of caries for the shortest period of time.

It is also this study in Bauxite that brought about the discovery of fluorine, an unexpected constituent of water supplied. We owe this discovery, as mentioned before, to H.V. Churchill, chief chemist of the Aluminum Company of America.

The fluorine content at Bauxite was 14 ppm and was reflected in the general severity of the enamel lesion. Subsequent analysis of the water supply in other communities where mottled enamel existed, disclosed the presence of fluorine in variable amounts. Where no mottling existed, the water was found to contain only trace amounts or to be totally free from this element.

Ten years later, after a change of water supply in these communities, a re-examination disclosed no new fluorosis among children born after the change.

The important conclusions to be drawn from these studies are:

1 - That dental fluorosis can be acquired only during enamel calcification, and that once acquired, it is permanent;

2 - That an individual whose teeth are completely calcified will not acquire dental fluorosis by residing in a locality where fluorine is present in the water supply, however high the concentration.

Further studies brought out the fact that caries immunity was co-existant with dental fluorosis however severe.

It is this observation that attracted the attention of Dean and co-workers. The problem was to determine the amount of fluoride content a water supply should have, to be able to afford protection against dental caries and at the same time be low enough to avoid conspicuous dental fluorosis.

In other words, here was a substance, fluorine, a trace element in nutrition and a natural constituent of water which, when found in excessive quantities (5 ppm or more) in drinking water, produced a pathological condition of the enamel, and at a reduced dosage (1 ppm) gave partial caries immunity. In both instances, the only requirement was that use of the water for drinking purposes last throughout the full period of enamel calcification. If this condition was not fulfilled, no dental defect (fluorosis) nor caries immunity resulted.

Dean demonstrated in 1938 an inverse variation between endemic dental fluorosis and dental caries experience. The group serving for the study consisted of 236 nine-year-old children of verified continuous residence in six different localities. It was established that a higher percentage of caries-free children was found among the users of higher fluoride domestic waters, than among those using a lower fluoride content. This caries immunity was present in deciduous as well as permanent teeth, whether or not the child showed microscopic evidence of mottled enamel. (15)

Several other studies were made in South Dakota and the Wisconsin cities of Green Bay and Sheboygan, WISCONSIN, that further substantiated this observation. The Galesburg-Quincy study by Dean and Al in 1939 lent much additional support to the "fluorine-dental caries" hypothesis; the Galesburg children, using throughout life a domestic water containing 1.8 ppm of fluoride, experienced only about one third as many caries as the Quincy children using a fluorine-free water. (Mississippi River).

An outstanding observation in the course of this study was the caries experience in the anterior teeth. The Macomb-Quincy

children, using fluoride-free water, experienced sixteen times as much of this type of caries as the Galesburg-Monmouth children using fluorine concentrations of 1.8 ppm. From these studies, an important epidemiologic observation may be drawn: "that limited immunity-producing factor present in the water is operative whether or not the tooth is affected by mottled enamel." (See Table II and graph)

It was necessary, however, to submit this naturally occurring phenomenon to various kinds of verification under different existing conditions. This was done through the course of the same study, what we may call Dean's survey of twenty-one cities in four different states: Elmhurst, Maywood, Aurora, Joliet, Elgin, Evanston, Oak Park and Waukegan, Illinois; Zanesville, Portsmouth, Middletown, Marion and Lima, Ohio; Elkhart and Michigan City, Indiana; Colorado Springs and Pueble, Colorado; Quincy, Galesburg and East Moline, Illinois.

Here was a number of cities with a real wide variety of water supplies, different, not only in fluoride content, but in other minerals as well. The range of supply was wide and varied, coming from Lake Michigan, the Mississippi, the Ohio and Arkansas Rivers, from deep wells of different fluoride concentration and total hardness. Colorado Springs obtained its source from snow water from high on Pike's Peak (2.6ppm). The fluorine concentrations were from 0.1 ppm to 2.6 ppm.

It would be too extensive and quite unnecessary to relate all the details of these investigations. However, it may be stated again that analysis of these data revealed that children using water with as little as 1 ppm fluorine experienced only about a third as much dental caries as other comparable groups using non-fluoridated water.

12 to 14-year-old children were drawn from the four states and formed comparable groups. The groups were tabled and examined according to the concentration in fluorine of the public water supply.

Group No. 1. Composed of 847 children using water with 1.4 ppm fluorine or more averaged 2.4 D.M.F. (a) teeth per child;

Group No. 2. Composed of 1403 children using water with fluorine concentration of 1.0 ppm to 1.4 ppm showed 2.9 D.M.F. per child;

Group No. 3. Composed of 1140 children using water with 0.5 to 0.9 ppm fluorine had an average of 4.2 D.M.F. per child;

Group No. 4. With 3,867 children in the survey using water fluorine-free or with less than 0.5 ppm the evidence showed 7.4 D.M.F. teeth per child.

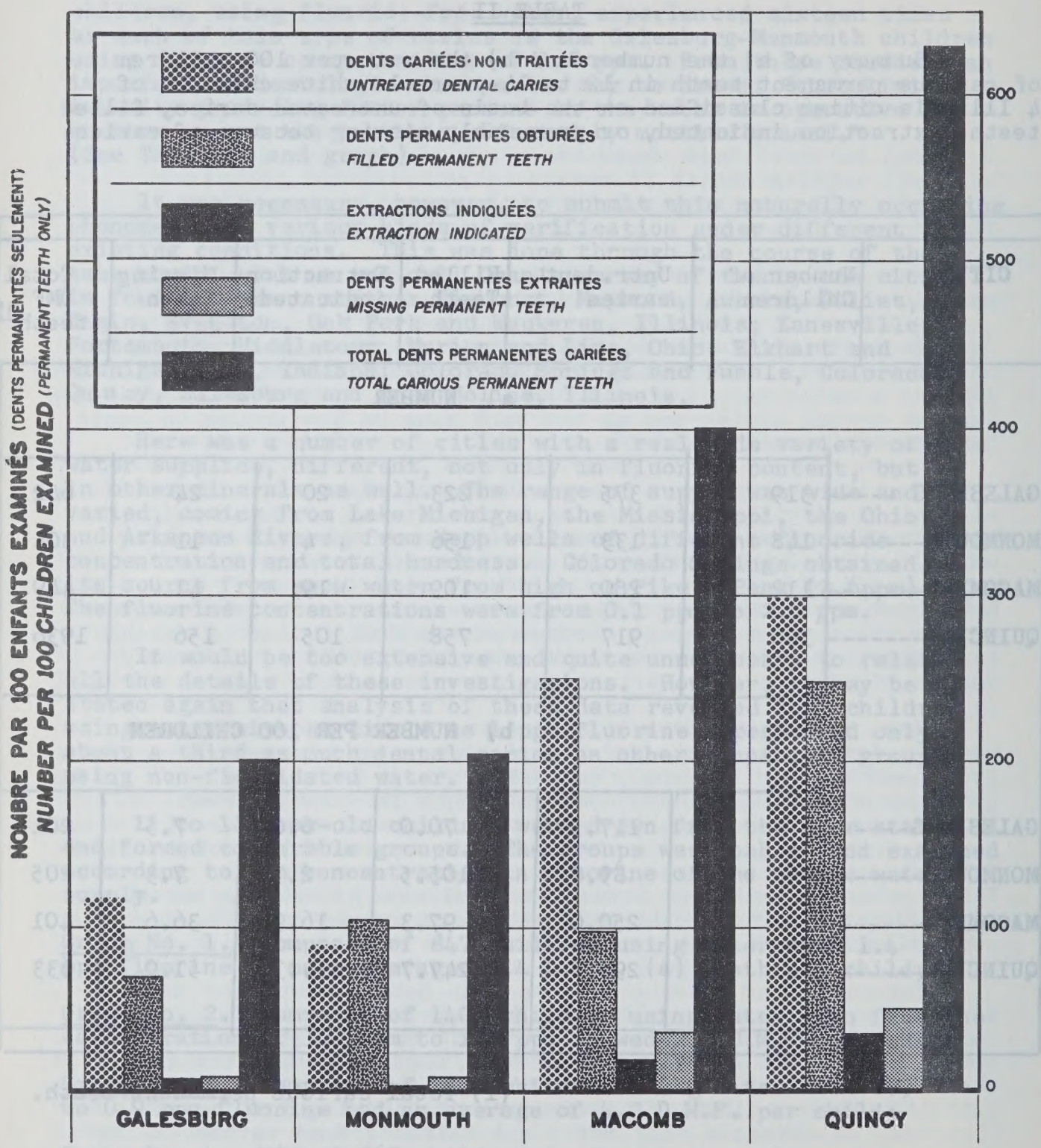
(a)D - Signifies Decayed teeth
M - Signifies Missing teeth
F - Signifies Filled teeth

TABLE II

Summary of a) the number and b) the rate per 100 children of carious permanent teeth in 12 to 14-year-old white children of 4 Illinois cities classified on the basis of untreated caries, filled teeth, extraction indicated, or presumably missing because of caries.

CITY	Number of Children	Untr.dent. caries	Filled Teeth	Extraction indicated	Missing Teeth	Total DMF teeth(1)
a) NUMBER						
GALESBURG -----	319	375	223	20	24	642
MONMOUTH-------	148	133	156	4	11	304
MACOMB---------	112	280	109	19	41	449
QUINCY---------	306	917	758	105	156	1936
b) NUMBER PER 100 CHILDREN						
GALESBURG-----		117.5	70.0	6.0	7.5	201
MONMOUTH------		89.8	105.5	2.7	7.3	205
MACOMB--------		250.0	97.3	16.9	36.6	401
QUINCY--------		299.6	247.7	34.3	51.0	633

(1) Total carious permanent teeth.


DENTS CARIÉES NON TRAITÉES
UNTREATED DENTAL CARIES
DENTS PERMANENTES OBTURÉES
FILLED PERMANENT TEETH
EXTRACTIONS INDIQUÉES
EXTRACTION INDICATED
DENTS PERMANENTES EXTRAITES
MISSING PERMANENT TEETH
TOTAL DENTS PERMANENTES CARIÉES
TOTAL CARIOUS PERMANENT TEETH
NOMBRE PAR 100 ENFANTS EXAMINÉS (DENTS PERMANENTES SEULEMENT)
NUMBER PER 100 CHILDREN EXAMINED (PERMANENT TEETH ONLY)
600
500
400
300
200
100
0
GALESBURG
MONMOUTH
MACOMB
QUINCY

Children examined	D.M.F.	P.P.M.
3,867	7.4	0.5
1,140	4.2	0.5 - 0.9
1,403	2.9	1.0 - 1.4
847	2.4	1.4

This is in reality the study that permitted Dean to finally establish at 1 ppm the optimum concentration of fluorine required to give maximum dental caries immunity with a minimum degree of fluorosis.

So far, we can summarize by stating that the epidemiologic investigations give conclusive evidence that fluorine is the etiologic agent in the production of fluorosis, that fluorosis is co-existant with dental caries immunity, and that fluorine is water-borne; that the studies in Bauxite, Britton and Colorado Springs demonstrated that this element was effective during the period of enamel calcification; that the severity of the mottling and the percentage of individuals affected are correlated with the concentration of fluorine in the water and the amount ingested. Therefore, in establishing a fluoridation program, one must take into consideration the climate, the average annual temperature, the relative humidity.

For example, in a region where the average temperature is between 70°F and 80°F., the concentration of fluorine would be less than in another with an average annual temperature of 60°F. It has been suggested that an average annual temperature of 60°F., or less requires 1 to 1.2 ppm fluorine, in order to obtain the full prophylactic effect. In warmer climate, the concentration may be down to 0.7 ppm.

We know also that fluorosis becomes discernable with concentrations 1.5 ppm. Further study of this phenomenon revealed that 12 to 14-year-old children who have been using throughout life a concentration as low as 1 ppm, experience approximately one third as much dental caries as children of similar age using fluorine-free water. Evidence also was presented showing that this caries inhibitory effect remains operative at higher age levels. (16)

Fluorine metabolism

Fluorine ingested through drinking water is taken up by the teeth and bones. At the start, retention may amount to as much as 50% of the intake, but gradually, a balance is established and results in the excretion through the urine of as much as 90% of the daily intake.

In studying the effects of fluorine in body tissue, McClure expressed the opinion that "there is a very efficient elimination of fluorine by way of the urine, and also some loss of fluorine by way of body sweat, and possibly through the insensible cutaneous perspiration.

"The results of fluorine balance studies are interpreted as being strongly indicative that quantities of fluorine up to 3-4 mg ingested daily are perhaps more than 90% eliminated by the average adult. The hazard of cumulative toxic bone fluorosis, surrounding most domestic fluoride waters, now seems greatly reduced by the observation that a remarkably efficient excretion of fluorine is a normal kidney function." (17)

If fluorine is excreted through the urine, it is necessarily carried to the kidney through blood circulation; it then becomes logical to wonder whether raising the concentration of a water supply to 1 ppm will result in an increase of fluorine in the circulating blood.

An investigation carried out by Smith, Gardner and Hodge showed that if a fluoride concentration in the water of 0.06 was carried to 1.36 ppm (23 fold increase) the mean urinary fluoride increase was 0.06 to 1.12 ppm (10 fold increase) while the average blood concentration increases from 0.014 to 0.040 ppm (3 fold increase). (18) Consequently, it can be said that the higher level of fluoride in the water supply does not produce dangerously high blood fluoride concentrations.

Evans and Phillips (31), reviewing the evidence of fluorine as influencing the course of hyperthyroidism, analyzed thyroids of forty thyroidectomy cases for fluorine and iodine and compared the findings with the basal metabolic rates. There was no evidence drawn from the data obtained that fluorine in any way, played a part in human hyperthyroidism by its action on the thyroid gland.

Other instances show that there might be some beneficial effects on hearing. Lewy (32) found hearing defects in 4.9% of 109,869 children from low fluoride districts in Illinois, and 2.8% in 20,488 from districts with fluorine not over 1.4 ppm.

Toxicity of fluorine

It has been shown in several instances that the only deleterious effect of fluorine concentrations in drinking water was evidenced by mottled enamel. In fact, nature itself provided us with the necessary evidence, when it was shown that communities, without even being aware of the fact, had been consuming for years water with as high as 15 ppm of natural fluorine. The only deleterious effect shown was an unsightly mottling of the enamel tissue of the teeth.

The first and most sensitive indication of an adverse physiological effect of fluorine ingestion is dental fluorosis. Experience shows that the prolonged ingestion of drinking water with a mean concentration of fluorides below the level causing dental fluorosis has no harmful effect.

It was suggested that a possible increase in bone fracture would result from fluorine ingestion through communal waters. In a study by McClure (33), on the relation of fluoride from water supplies to height, weight and bone-fracture experience of 1,458 high school boys and 2,529 young men taking physical examinations at United States armed forces induction centres, no relation was found between fracture experience and fluoride exposure.

Studies have also tended to relate cancer, particularly breast cancer to excessive fluorine intake. Investigation in communities with high fluorine concentrations in the water supplies proved these theories to be altogether unfounded. Those desiring further enlightment regarding the toxic effects of fluorine should refer to a paper given at the American Water Works Association Convention in Montreal, May 26th, 1952, by Dr. Adélard Groulx, M.P.H., Director of the Department of Health of the City of Montreal: "Fluoridation and the Health Officer." (19) The lethal dose of fluorine is from 4 to 5 gms.

Acute morbidity manifested by increased salivation and vomiting may be caused by 0.25 gm of sodium fluoride. In 0.25 gm of sodium fluoride we have 0.12 gm of fluorine.

A quart of 1 ppm fluoridated water contains one milligram of fluorine and it would be necessary to drink 120 quarts of water in a relatively short time to absorb 0.12 gm of fluorine; or to put it another way, this quantity (0.25) in an 8-oz. glass of water represents 1000 ppm sodium fluoride, or about 450 ppm fluorine; to obtain this concentration, it would be necessary to add 4 tons of sodium fluoride for every million gallons of water treated, which obviously is not possible in a water fluoridation program.

Fluoride mechanism

The exact mechanism through which fluorine compounds operate in the reduction of dental caries is not yet clearly understood. That fluorine intake is the sole index of dental caries prevalence, I do not want to infer. Too many other contributing factors are involved; but that fluorine intake is a mean of partial control, there is no doubt. We must admit the necessity of a fluorine, calcium, phosphorus balance in the formation of the teeth, if we want to produce caries immunity. So far, research has suggested three hypotheses:

1 - Fluoride lowers the solubility of the tooth structure;

2 - Fluoride inhibits the bacterial or enzymatic processes that are believed to dissolve the protein and calcified substance of the tooth;

3 - Fluoride changes the bacterial flora of the mouth, thereby reducing the number of acidogenic bacteria that are associated with the caries process. (20)

Numerous studies have been made, and bacteriological findings as measured by lactobacilli counts, indicate an increase of lactobacilli in the oral flora of children using fluorine-free water as compared to children residing in a fluoride area.

Experiments on the solubility of the enamel have been made and gave evidence of a definite modification of the enamel surface treated with sodium fluoride for periods as short as three minutes.(21)

The hardness of the enamel has been shown to be correlated to the degree of its fluoride content. Research on experimental dental caries in rats and hamsters has been extensive and the reader is referred to the report by Shour and Smith (22) and a bulletin by McClure (23) which was published in 1939.

The period at which the fluorine is acquired by the tooth may make a great difference in the action of the fluorine on the tooth substance. Although evidence shows that fluorine is active during tooth formation, several studies have tended to show the possibility of caries immunization through topical application.

Topical application

The evidence that was brought forth through the epidemiological studies on the relationship of fluorine to dental tissues suggested the hypothesis that application of fluoride solutions to the external surfaces of the enamel might possibly decrease the tooth's caries susceptibility. This hypothesis served as a basis for extensive laboratory and clinical research. The first reports on the value of topical application were controversial, but a series of studies by Knutson of the U.S.P.H.S. (36) seems to have pointed out that a definite reduction of 40% in dental caries experience can be expected, provided the proper technique is applied.

Technique of application

1 - The first step in the application of this prophylactic measure, is the cleaning of the teeth; this is necessary, if we want to remove the debris from the crown surfaces and the interstices, and good care must be taken so that no deposits remain in the pits and fissures: the importance of this pre-treatment measure is brought forth by the results of extensive clinical testing and laboratory findings of Adler and Straut (37) which indicate that this initial cleansing doubles the effectiveness of the series of applications.

Ghrietzberg (38),however, demonstrated that cleansing of the crown surfaces of the teeth can be accomplished as effectively by supervised tooth brushing as by the rubber cup and pumice method.

2 - The teeth must then be isolated so as to block off the flow of saliva; in most instances, it is necessary to use the saliva ejector.

3 - Each tooth must then be dried under a 20 to 30 lbs air pressure. This step also is important, because it has been proven that the effect is doubled when the solution is applied to a dry tooth.

4 - The solution used is a 2% solution of sodium fluoride in distilled water. This solution is unbuffered and stored in ordinary glass bottles. It is applied to the teeth and permitted to dry in air for about four minutes. Under those conditions of storage, it has been found that the pH of the solution increases from neutral to 9.5 in a period of approximately six months. (40) Laboratory results seem to favour an acid solution with a pH of 4.0 to 5.5, but clinical evidence has shown that an acid solution should not be used because it is not effective. (41)

The period of drying of the solution on the tooth is determined by the time limit which is approximately four minutes. Therefore, it is easy to see that it is not the drying of the solution on the tooth, but the time of exposure of the tooth to the solution which is important.

These four steps must be followed faithfully if we do not want the effect of the fluoride applications to be jeopardized. Four applications, (one a week), are necessary, and should be given every three years in order to protect the new teeth as they come into the mouth. The tentative ages at which this treatment should be given are 3, 7, 10 and 13.

Topical application of a 2% solution of sodium fluoride as a preventive measure of caries inhibition on a mass basis has been recommended by the Council on Dental Health and the Council on Dental Therapeutics of the American Dental Association, The Michigan Workshop on the Evaluation of Dental Caries Technics, the Dental Section of the American Public Health Association, the Association of State and Territorial Health Officers.

The measure was sponsored by the United States Public Health Service and the Children's Bureau, when Congress of the U.S. appropriated one million dollars in 1948 for a nation-wide topical fluoride demonstration program. (35)

Water fluoridation

As knowledge of the importance of the fluorine ion as a factor in the inhibition of dental caries extended, public health interests were awakened.

Would the addition of fluorine, in a verified concentration, to a fluorine free-water supply give results similar to those found in

communities where the people benefited from naturally fluoridated drinking water?

Several pilot programs were undertaken to verify this hypothesis, the most important being carried out in Grand Rapids, Michigan, Newburgh, N.Y., and Brantford, Ont. All three were conducted in the same manner; each having a control city, identical age groups, with a sufficiently large number in the experiment to formulate significant results.

Methods were used to eliminate examiner bias. The same fluoridating agent, sodium fluoride, was used in all three experiments. After five years of controlled studies, all three showed an appreciable reduction in dental caries experience.

Newburgh-Kingston Experiment (24)

Both these cities situated in upperstate New York have been experimenting with water fluoridation since 1944. Each have an approximate population of some 30,000. Habits, living and economic conditions are approximately the same in both communities.

In addition to a complete dental examination with mouth mirror and explorer of some 3,400 Newburgh children and 2,810 Kingston children 6 to 12 years old, data on physical examinations, laboratory tests and roentgenographic examinations of the hands, forearms and tibias of approximately 500 children in each city were made.

In 1949, a first progress report showed that, in regard to the medical aspects of the study, the results showed no deleterious systemic effects from the ingestion of fluoride in drinking water in the dosage employed (1 to 1.2 ppm). In regard to the caries experience, there was a consistent downward trend in D.M.F. permanent teeth of 6 to 12-year-olds, in other words, a drop of 21 to 15 D.M.F. per 100 permanent teeth representing already, after three years, a 30% reduction.

In Newburgh, 2,810 children were examined in 1944-45, while 2,553 Kingston children were examined in 1945-46 with a respective D.M.F. rate per 100 erupted permanent teeth of 20.6 and 20.2 with a relative difference of 2% shown in the higher Newburgh rate.

In 1950-51, 2,410 Kingston children examined gave a D.M.F. of 21.7 per 100 permanent teeth, while in Newburgh where 2,709 children were examined, the D.M.F. was 13.0, indicating a relative difference of 40% due to a lower caries experience in Newburgh during the five-year period. The children considered in this analysis are in the 6 to 12-year age agroup. (See Table III)

Analysis was also presented for deciduous teeth in children, ages 5 to 8 inclusive; the following table indicates the results: (See Table IV)

TABLE III

Age adjusted[1] DMF[2] Rates per 100 erupted permanent teeth of children ages 6 - 12 inclusive, in Newburgh[3] N.Y. (fluoride city), and Kingston, N.Y. (control city).

1944 - 1951

CITY	YEAR	CHILDREN EXAMINED	DMF per 100 ERUPTED PERMANENT TEETH	RELATIVE DIFFERENCE
KINGSTON	1945-46	2,553	20.0	
NEWBURGH	1944-45	2,810	20.6	Newburgh rate 2% higher than Kingston rate
KINGSTON	1950-51	2,410	21.7	
NEWBURGH	1950-51	2,709	13.0	Newburgh rate 40% lower than Kingston rate

(1) Age adjusted to the permanent tooth population in Kingston in 1945-46

(2) DMF includes teeth decayed, missing (lost subsequent to eruption) or filled

(3) Sodium fluoride added to water supply beginning May 2, 1945 to increase concentration to 1.2 ppm.

TABLE IV

def[1] rates per 100 deciduous teeth present, in children ages 5-8 inclusive, in Newburgh[2], N.Y. (fluoride city) and Kingston, N.Y. (control city)

1944 - 1951				
CITY	YEAR	CHILDREN EXAMINED	def per 100 DECIDUOUS TEETH PRESENT	RELATIVE DIFFERENCE
KINGSTON	1945-46	1,345	35.3	
NEWBURGH	1944-45	1,426	37.0	Newburgh 5% higher def rate than Kingston rate
KINGSTON	1950-51	1,414	30.8	
NEWBURGH	1950-51	1,643	20.1	Newburgh 35% lower def rate than Kingston rate

(1) def includes deciduous teeth decayed, indicated for extraction or filling

(2) Sodium fluoride added to water supply beginning May 2, 1945 to increase concentration to 1.2 ppm.

TABLE V

Children with caries-free deciduous cuspids, first and second molars in children, ages 5-6 inclusive, in Newburgh[1] (fluoride city) and Kingston, N.Y., (control city).

1944 - 1951					
CITY	YEAR	NUMBER OF CHILDREN[2]	NO. CHILDREN[3] CARIES-FREE	PER CENT CHILD CARIES-FREE	RELATIVE DIFFERENCE
KINGSTON	1945-46	602	132	21.9	
NEWBURGH	1944-45	677	132	18.2	Newburgh rate 17% lower than Kingston rate
KINGSTON	1950-51	524	157	30.0	
NEWBURGH	1950-51	730	359	49.2	Newburgh rate 64% higher than Kingston rate

(1) Sodium fluoride added to water supply beginning May 2, 1945 to increase concentration to 1.2 ppm.

(2) Children with all 12 specified teeth present.

(3) Children with all 12 specified teeth caries-free.

An interesting aspect is presented in Table III regarding children with free deciduous cuspids, first and second molars, ages five and six.

It has always been thought that fluorine does not penetrate the placental barrier if the ingestion is from a solution of less than 5 ppm. However, if we study the percentage of caries-free children of 5 and 6 years of age, we will note that in 1944-45, the percentage was 18.2 in Newburgh, rising to 49.2 in 1950-51, showing an increase in caries-free children of over 64%, if we compare it to the Kingston rate. (See Table V)

This is, however, quite difficult to explain, since it is known that fluorine is beneficial during the formative period of the teeth. We know that these 5 and 6-year-old children examined in 1950-51 were just about born in the experiment. We also know that at birth, the crowns of the deciduous cuspids and molars are partly calcified. We also know that the deciduous teeth have been exposed to decay for a much longer period of time than the 6-year-molars which are generally the basis for the study at this phase of the experiment.

It would then seem that it is not necessary that fluoridation be applied during the whole calcification period, to obtain full prophylactic measure.

It also explains the fact that older age groups also obtain appreciable benefits although they were not born in the experiment. This seems to be worthy of a more thorough investigation.

Although the Newburgh-Kingston experiment was initially slated as a 10-year study, the data presented after 5 years of fluoridation show a consistent downward trend in the D.M.F. rates among permanent teeth of 6 to 12-year-old children in Newburgh, as compared to the city of Kingston, which shows no changes. The reduction in Newburgh is approximately 32.5%. The number of caries-free first permanent molars among 6 to 9-year-old Newburgh children increased from 58.9 to 76.9, and for the 10-12-year group, from 23.8 to 32.5. In Kingston, there was no change. The number of children with their deciduous teeth completely caries-free showed an increase three times as great in Newburgh as in Kingston.

Data presented in 1951 brought further enlightment as to the effectiveness of fluoridation in caries control among the Newburgh child population. The Newburgh experience was plotted against the Aurora curve where the population has had the benefit of natural fluorides at 1.2 ppm. for a number of years. It is evident at a glance that the addition of fluorides have the same preventive effect as fluorine occurring naturally in water.

The consistent downward trend of the Newburgh curve is significant to the point that as each succeeding year in the

experience goes by, it is evident that after three more years of fluoridation, it will equal, if not better, the Aurora line. (See graph Newburgh-Kingston.)

This is of great public health significance as deductions from data collected would permit us to visualize a 65% decrease in dental caries and the possibility that we may cope with this problem which under present conditions, has grown out of proportion to the number of dentists available. The Newburgh as well as the Grand Rapids and Brantford studies have all been carried out under similar conditions with identical results.

The Grand Rapids Survey (25)

The Grand Rapids studies were carried out under the supervision of the U.S.P.H.S., with Muskegon, Michigan, selected as a control city. Here also, data were collected for comparison with the dental caries rates in Aurora, Ill., which has a naturally fluoridated water supply at 1.2 ppm.

Virtually, all the school population of the three cities underwent a detailed dental examination, those in continuous residence forming the base line information in this report.

The analysis of 1949, hardly 4 years after fluoridation, showed a mean 40% reduction of the D.M.F. rates; these results are similar to those obtained in Newburgh, and are probably more significant on account of the larger child groups where the teeth were calcified following fluoridation.

As I was told by Dr. F. Arnold of the U.S.P.H.S., who is actively interested in the study, a more recent report is under preparation at the National Institute of Research and should be of great interest to all those concerned with the problem.

The Dean-Arnold Study, Grand Rapids (See Table VI)

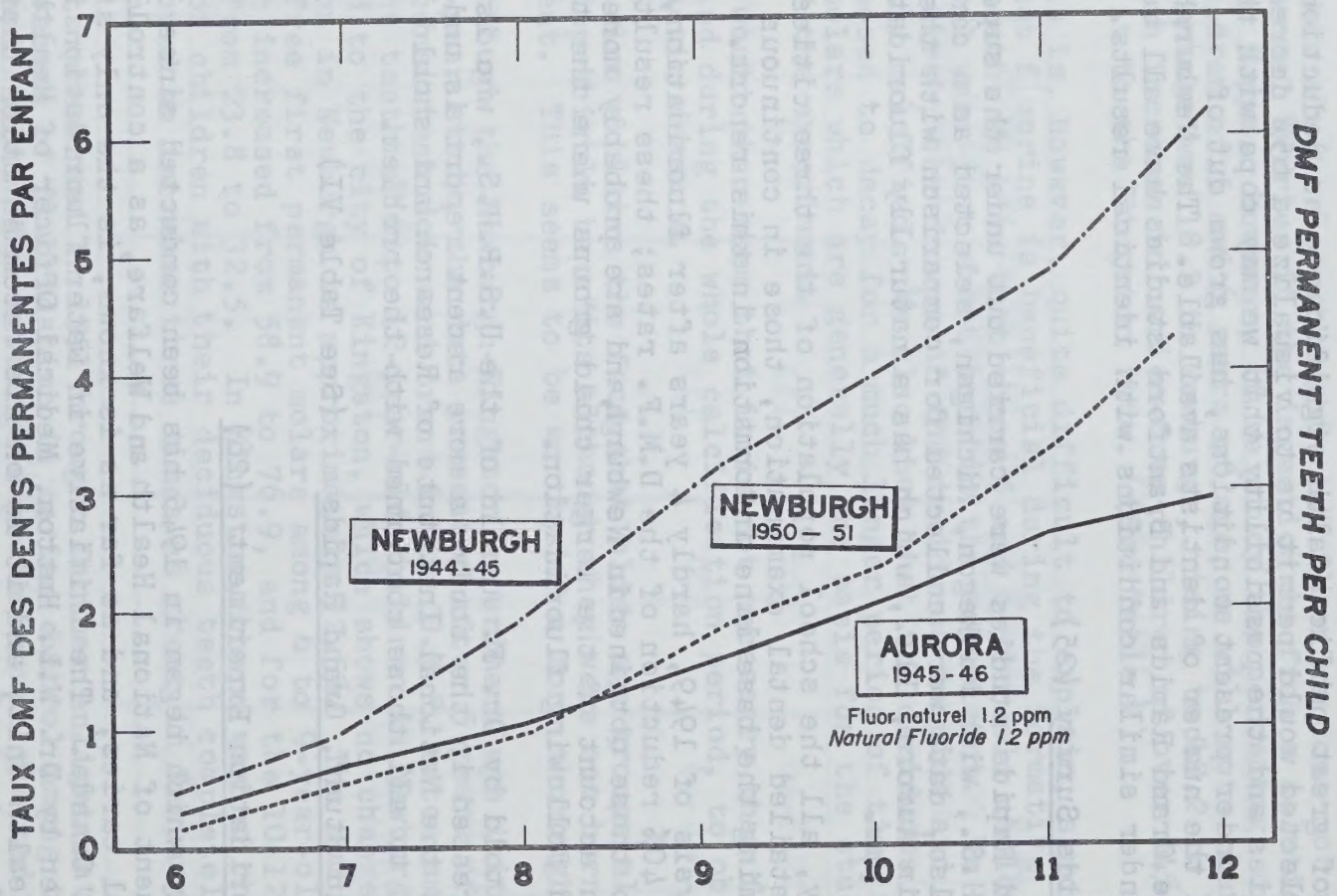
Brantford Fluoridation Experiment (26)

This study which began in 1945 has been conducted since 1948 by the Department of National Health and Welfare, as a controlled study of dental caries, and as far as is known, is the only study of its kind in Canada. The initiative in water fluoridation in Canada was taken by Dr. W.L. Hutton, Medical Officer of Health, Grant County Health Unit, and it is on his recommendation that sodium fluoride was added to the Brantford water supply in June 1945, hardly one month after the initiation of the Newburgh study.

The concentration was fixed and maintained between 1.0-1.2 ppm. Data were collected through an initial survey among the school

TAUX DMF[1] PAR ENFANT DES DENTS PERMANENTES- TABLEAU COMPARATIF D'UNE ZONE FLUORÉE ARTIFICIELLEMENT ET NATURELLEMENT. NEWBURGH[2], N.Y. ET AURORA, ILL., 1944-51.

DMF[1] PERMANENT TEETH PER CHILD IN CONTROLLED AND NATURAL FLUORIDE AREAS, NEWBURGH[2], N.Y. AND AURORA, ILL., 1944-51.



1) DMF comprend dents permanentes cariées, extraites ou à extraire. et dents obturées.

2) La fluoration par le fluorure de sodium à une concentration de 1.2 ppm commença le 2 mai 1945.

1) *DMF includes permanent teeth decayed, missing (lost subsequent to eruption), and filled.*

2) *Sodium fluoride added to water supply beginning May 2, 1945 to increase concentration to 1.2 ppm.*

population, and recorded annually since 1945.

Sarnia which had a fluorine-free water was selected as control, while Stratford, with a concentration of 1.3 ppm fluorine occurring naturally in its water supply, formed a basis for analysis between artificial and natural fluoridation.

The reduction in caries rate after five years of fluoridation was 31%; the change in permanent D.M.F. teeth was 44% on the average.

The present average of caries-free children, age 5 to 16, in 1944-45, was 5.2% and, in 1950, 15.6%, an increase after 5 years of fluoridation of 300%. A summary of Brantford School Children with perfect teeth, 1944-1951, is given on page "T-27". (See Table VII)

These figures cannot be overlooked and the value of water fluoridation has been fully established as a partial control of dental caries. Surely, we do not know as yet the full value of this prophylactic measure, as children entering the experience at birth in 1945 will have to wait until 1957 before the eruption of the 12-year molars; but the evidence at hand is already so convicting that it would be unfair to deprive those that are being born of the full benefits of water fluoridation.

According to Stadt's report on December 12th, 1951, 159 communities in 37 States with a total population of 4,175,259 have already initiated fluoridation programs (27). 136 others have approved this public health measure, while in 267 others, water fluoridation is under consideration. The total population involved is 33,743,894, nearly three times the population of our country.

According to Hill, Jelinek and Blayney, three million or more are using water containing natural fluorine at 0.9 ppm or more, and this figure may increase to four million, when more complete reports are available. (28)

If we consider that approximately 100,000,000 people are provided with potable water by 16,750 public water supplies, it is possible that by the end of 1953 around 40% of these people will be receiving water with natural or applied fluorides.

After a thorough study as to the feasibility of the measure, early in July, water fluoridation was applied in Washington, D.C., with an initial concentration of 1 ppm sodium silico-fluoride. It might be well, at this time, to summarize the evidence at hand in evaluating water fluoridation as a public health measure in the partial control of dental caries.

Most public health measures have been subject to criticism, and only after their effectiveness and value have been established have they reluctantly been accepted. In fact, one can recall that only a few years ago, irresponsible groups here, in our city, were

TABLE VI

Dental caries experience, permanent teeth, observed among 33,955 children, age 5-16, of Grand Rapids, Muskegon, and Aurora, expressed as DMF teeth per child with percentage reductions observed (continuous residents).

AGE	GRAND RAPIDS, MICHIGAN			AURORA, ILLINOIS		MUSKEGON, MICHIGAN		
	EXAMINATIONS MADE 1944-45	1949-50	PERCENTAGE REDUCTION	EXAMINATIONS 1945-46	PERCENT. LESS THAN G.R. 1944-45	EXAMINATIONS MADE 1944-45	1949-50	PERCENT. CHANGE
5-----	0.11	0.03	72.7	0.06	45.5	0.06	0.14	+133.3
6-----	.78	.38	51.3	.28	64.1	.81	.63	-22.2
7-----	1.89	.76	59.8	.70	63.0	1.99	1.43	-28.2
8-----	2.94	2.16	26.5	1.04	64.6	2.81	2.58	- 8.2
9-----	3.90	2.48	36.4	1.52	61.0	3.81	3.88	+ 1.8
10----	4.92	3.56	27.7	2.02	59.0	4.91	4.44	- 9.6
11----	6.41	4.69	26.8	2.67	58.4	6.32	5.93	- 6.2
12----	9.07	7.02	13.0	2.95	63.5	8.66	7.21	-16.8
13----	9.73	8.11	16.7	3.09	68.3	9.98	9.52	- 4.6
14----	10.94	8.90	18.6	3.64	66.7	12.00	11.08	- 7.7
15----	12.48	11.80	5.5	4.54	63.6	12.86	10.32	-19.8
16----	13.50	11.83	12.4	5.19	61.6	14.07	12.51	11.1

TABLE VII

YEARLY PERCENTAGES OF BRANTFORD SCHOOL CHILDREN WITH PERFECT TEETH. 1944-1951.

YEAR	NUMBER OF PUPILS	HAVING PERFECT TEETH	
		NO.	%
Pre-fluoridation 1944 & 1945	5,482	284	5.18
Post-fluoridation			
1946	2,787	200	7.17
1947	2,751	254	9.23
1948	2,863	356	12.44
1949	2,870	398	13.86
1950	2,873	449	15.63
1951	2,906	464	15.97

entertaining the idea of reintroducing non pasteurized milk, not recognizing that this public health measure had practically wiped out the milk-borne diseases, such as typhoid fever, Shigellosis, Brucellosis, enteric diseases, as well as many Streptococcal infections.

We are thankful for those measures which gave us water purification through chlorination, when we recall the cholera epidemics which swept through China, Asia, India, and which prevailed in England during the first half of the XIXth century. Yet, public health education was not sufficient. Public opinion was forced to accept control of this dreadful disease through water purification.

It is the duty of the public health officer to recognize the value of a public health measure and if its worth is well established, to see that it is applied.

In the study of dental caries, we are dealing with a disease which knows no age group. This disease is pandemic in nature; both young and old are its victims. The only time this disease can be checked is at the onset and more so between the ages of 3 to 9.

Statistics from the New York health department and the Gughenheim dental clinic show the average caries experience of pre-school children throughout the entire population, either rich or poor: at 2½ years, 48% have dental caries experience; at 3 years, 80% and at 5 years, 98.6%. When a child is between 6 and 7 years old, 7 teeth are involved; between 12 and 14 years, 12 teeth are involved.

Although 15% of all the dentists in the United States are in New York City, it would require 3,000,000 dentist-hours at ½ hour per patient to give all the city's 905,000 children partial dental care, which is impossible.

In Montreal, the needs are much greater due to lack of dentists and facilities for providing adequate dental care. Evidently, the same condition exists throughout the country.

This is sufficient to show that the only way to cope with the dental caries problem is through mass control. This opportunity is offered through water fluoridation.

Through studies and surveys that have been made, we know that certain communities whose water supply contains 1.0 ppm fluorine or more, the caries experience of the population is on the average two thirds lower than the caries experience of a nearby community whose water supply is fluorine free. Extensive data, some of which have been precedingly summarized, show the relationship between the amount of fluorine in the water and the degree of mottling in the enamel.

Dean's studies have also shown that where no mottling occurred, no fluorine was present in excessive doses in the water. He also

demonstrated that between 1.0 and 1.5 ppm fluorine, there was no mottling of an esthetic value.

Throughout the Galesburgh-Quincy studies, and others, he established the inverse relationship between dental fluorosis and dental caries experience. The controlled studies in Grand Rapids, Newburgh and Brantford, where sodium fluorine was added to the public water supply, show, after seven years, a remarkable decrease in dental caries experience undoubtedly due to the affinity of calcium for the fluorine ion released in the water supply.

There have been, and naturally so, many objections to water fluoridation; if this had not existed, the measure would not have been so widely publicized, and its effectiveness as to results would not have been so thoroughly investigated.

As it is, the United States Public Health Service, through its Institute of Dental Research, has been allocating enormous funds to research alone in water fluoridation.

Sound criticism is necessary; it promotes enthusiasm and interest. However, criticism must be well founded, so as not to neutralize the value of a measure of such public health significance.

We cannot overlook the fact that water fluoridation has obtained the approval of the major health institutions throughout the country, some of which are:

The United States Public Health Service;
The American Public Health Association;
The American Association of Public Health Dentists;
The American Medical Association;
The Canadian Public Health Association;
The Canadian Dental Association;
State and Territorial Dental Health Directors;
The American Dental Association;
The State and Territorial Health Officers, etc.

The sole fact that such authoritative bodies have definitely given their full approval of water fluoridation as a reliable means of reducing dental caries indicates the seriousness with which this problem has been approached, and the thoroughness of the research and investigations which have resulted.

The beneficial effects of water fluoridation are numerous; through the pilot studies carried out to date, the evaluation of results has shown that:

1 - Six times as many children will have no dental caries experience;

2 - 60% lower caries experience rate can be expected;

3 - There will be a 75% decrease in the first permanent molar loss (29)

4 - 90% reduction in tooth surface caries in upper incisors. (30)

As a public health dentist, I cannot refrain from supporting such a far-reaching public health program. The evidence in its favour is tremendous, and as I was told in a personal interview by Dr. Ast, Director of Dental Services for the State of New York and initiator of the Newburgh-Kingston study, "fluoridation of Communal water supplies is here to stay, whether we like it or not."

It means to the generation born in the experiment a 65% reduction in dental caries. It means to each child one decayed tooth instead of three; it is equivalent to tripling the number of dentists actually available.

At present, hardly one-fourth of the increment of caries can be handled. This can be largely reduced through fluoridation without affecting the overall amount of actual restorative dentistry in the least.

It is not only the public health dentists, but the whole dental profession's responsibility to promote fluoridation, as it is their responsibility to improve the oral health of their respective communities.

A public health measure such as the fluoridation of the Montreal water supply demands the co-operation of the medical and dental societies, if we are to have a well-informed public.

It is the duty of every physician and dentist to supplement his present knowledge with the factual information now available in order to counter-balance the false statements that are being issued now and then by public-minded but irresponsible persons.

All those connected with dental, medical and engineering groups must be able to answer these questions concerning fluoridation: Is it harmful? - How much will it cost? - What will it do? - How do you do it?

It must be well understood by all that it is mostly the generation that is being born in the experiment that will benefit mostly from water fluoridation, the caries-preventive effect of adequate fluorine intake is conferred upon children up to the 12th year of life; in other words, during the calcification of the permanent teeth. There is, however, full reason to believe that this protection is extended throughout adult life to an appreciable degree (42); there is a safe margin between the trace amounts in drinking water which are required for optimal dental health and the amount which produces dental fluorosis.

The cost of fluoridation varies between .05 and .15 cents per capita, depending on the size of the community, the fluoridating agent which is used, the amount of fluorine in the water before

fluoridation, the type of equipment used, the number of installations required. By using sodium silico-fluoride instead of sodium fluoride, the overall cost would be cut by half.

Let us suppose that the average life span of an individual is 70 years, and the cost of fluoridation is .10 per capita. This would mean that to protect him by two thirds against what would have been his normal caries experience will cost $10.00; just about the cost of three ordinary amalgam fillings. Economically speaking, this cannot be underestimated.

The planning and estimation of the cost of fluoridation for any community is the job of the engineering department. I have, however, taken the liberty, basing my findings on our maximum daily consumption, of estimating what would be the actual cost of fluoridating the Montreal water supply. These figures are only an approximation and must not be taken at their exact value. The cost of equipment as well as the cost of the chemical used is subject to frequent fluctuations; the figures, as stated, are based on prices prevailing in the United States on May 1st, 1952.

Montreal draws its water supply from the St. Lawrence River, the only inlet being at Ville LaSalle. The maximum intake capacity is 300,000,000 gallons per day; the one point of treatment is the filtration plant located 5 miles from the intake with a pumping capacity of 350,000,000 Imperial gallons per day. This latter figure is used as the basis of estimation and is equivalent to 435,500,000 United States gallons, the raw water being of medium hardness.

The recommended chemical is Sodium Silico-fluoride (Na^2 Si F^6) as its present cost per quantity needed to treat one million gallons of water with 1 ppm fluoride is about 36% that of sodium fluoride and 27% that of hydrofluosilicic acid ($H^2Si\ F^6$) the other two fluoride ion compounds presently accepted for use in water treatment. The present unit price per pound of sodium Silico-fluoride is about $0.08 cents.

Two dry feeding devices of the gravimetric type are necessary; they should be complete with filling hoppers, chutes and dust collecting devices; the machine's capacity should be up to 5,000 pounds per 24 hours. The complete installation should include a metering device to control feeder delivery in proportion to flow through the plant.

Based on adding 1.2 ppm fluoride ion to the supply (a quantity estimated as necessary for a mean annual temperature between 60° and 70° F.) of 437,500,000 U.S. gallons, 7,300 pounds daily of sodium silico-fluoride will be required. However, the actual plant output may indicate a lower figure. This is equivalent to 2,664,000 pounds per year or 222,000 pounds per month.

Regarding the space requirements, the floor space (on two floors - one directly above the other) for the feeders, should be about 13 square feet per feeder on each floor; e.g., the feeder hopper must be on a floor directly above the feeder which occupies a floor space of approximately 38" x 50".

Sodium Silico-fluoride is available in 100 pound bags which have a dimension of 17" x 25" x 6½" or 1.6 cubic feet. A month's supply of chemical would, therefore, require (2,263) x 1.6 or 3620.8 cubic feet.

In a room with a ceiling height of 8 feet, a floor space of 452.6 square feet, or 20' x 23' will be required. Storage in a railroad box-car on a siding could be considered; it must be remembered however, that the chemical must not be subjected to dampness or humidity, if at all possible.

The initial cost of equipment exclusive of chemical storage space should not exceed $30,000.

The annual operating cost is estimated as follows:

Chemical	$124,000 (x)
Interest	1,200
Operation	7,000
	$132,200

However, this figure is based on the maximum plant output capacity and not on the average daily consumption.

As this is a maximum of 205,000,000 Imperial gallons per day (256,250,000 U.S. gallons per day), the cost of the chemical would be $71,000 per year and the chemical storage space would be proportionately lower.

In other words, the annual operating cost would be:

$71,000.00
1,200.00
7,000.00

$79,000.00 or less than $0.07 cents per capita. As stated before, these figures are personal estimates and may vary, according to certain unstated requirements and possible fluctuations, in costs of equipment and chemicals used. For instance, if sodium fluoride was the chemical used, this figure would probably be doubled and would indicate a cost per capita of approximately $0.11.

(x) based on maximum plant pumping capacity

Whichever way, we look at it, water fluoridation is just about the cheapest public health measure per individual treated ever applied.

Throughout the United States and Canada, the public, recognizing the prevalence of dental caries and that protective measures can be taken on a community-wide basis, are becoming gradually interested in the results of water fluoridation.

In this instance, it is the public health dentist and the sanitary engineers who, working together, have advanced us another step in the control of disease in man.

CONCLUSIONS

The effects of fluoridation of water on the fully calcified teeth of adults are still a topic for discussion, but as far as children are concerned there is no doubt that the incidence of dental caries can be reduced by approximately two-thirds.

It is true that objections are raised now and then to the adoption of the measure. Some people naturally timid object to anything that has never been done before. This is a natural reaction of certain individuals who are always afraid that if a change is attempted in their way of life, something terrible may happen. We know that certain concentrations of fluorine may cause mottled enamel but that a concentration of 1 ppm does not. We know beyond doubt that over 3,000,000 people in the United States have been consuming all their life naturally fluoridated water with much higher concentrations sometimes as high as 5.5 ppm (Bauxite 14 ppm) with no harmful effects other than mottled enamel.

However, one of the chief responsibilities of the public health officer is the prevention of disease; it is he who judges that the techniques utilized are designed to accomplish this purpose as effectively as possible. He may look back with satisfaction and consider the trend of certain communicable diseases that only a few decades ago accounted for high mortality, and have now all but disappeared. Typhoid fever, diphtheria, and scarlet fever are under control and are no longer public health problems. This is the result of our increase in knowledge of control methods and the application of new techniques.

Dental caries, however, the most prevalent of the chronic diseases still affects practically the entire population and until recently, the only protection against tooth mortality was the technical intervention of the dentist. Dental health today is recognized as an important part in the generalized public health program and for this reason, better methods had to be found for its control. If we consider the number of dentists available, it is evident that only a fraction of the population can and will be

treated. Thus, it is clear that the annual increments of untreated dental caries, especially among the child population, will only add to an accumulation of the disease.

Limited funds and personnel force the public health officer to decide what part of the program he will attack, since an all-out treatment program is impossible. For this reason, public health dental programs are confined almost exclusively to the child population.

It is with satisfaction that he reviews the progress made in recent years along the line of caries prophylaxis.

For this reason, the evaluation of the effectiveness of water fluoridation programs are of the utmost importance and the brilliant epidemiological studies in this field constitute a milestone in public health.

Water has become again a standardized vehicle in the prevention of disease. And we may now contemplate the possibility of visualizing the next generation of children attaining adulthood with a full complement of teeth.

Much of the information included in this paper is the result of personal interviews with Dr. F.J. McClure, National Institute of Dental Research; Dr. F.A. Arnold, National Institute of Dental Research; Dr. Harry Strusser, Director, Bureau of Dentistry, N.Y. City; Dr. H.K. Brown, Chief, Dental Division, Department of National Health and Welfare, Ottawa.

To these gentlemen, I am deeply grateful.

To Dr. Adélard Groulx, M.P.H., Director of the Department of Health of the city of Montreal, I give my sincere thanks for giving me the opportunity of writing on such a fascinating topic as water fluoridation.

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APPENDIX "T"

FLUORIDATION AND THE HEALTH OFFICER

by

Dr. Ad. Groulx, M.P.H.,
Director, Department of Health,
City of Montreal.

Conclusions.

For many generations, 3,000,000 people in the United States, lived normally in districts where fluorine concentrations were higher than those recommended to prevent dental caries. The only discovery made by research workers is an enamel defect called dental fluorosis, due to excessive concentration of fluorine, but without any ill physiological effects.

The Council on Pharmacy and Chemistry and the Council on Foods and Nutrition of the American Medical Association issued a joint statement regarding the fluoridation of community water supplies. In the opinion of both Councils the addition of one part per million of fluorine to drinking water approved methods of control is harmless.

The only difficulty so far revealed is a possible increase in mottling of the tooth enamel. From the available evidence based on thousands of observations the incidence of mottling of the enamel in children who drink water containing fluorine up to a concentration of one part in a million is minimal and detectable only by careful dental examination.

Evidence of toxicity other than the effect on enamel has not been reported in communities where the water supply has several times this concentration. After considering the evidence available at this time, the Councils believe that the use of drinking water containing up to one part per million of fluorine is safe. (However, the use of products which are naturally high in fluorine content, such as bone meal tablets, or of lozenges, dentifrices, or chewing gum to which fluorine has been added, should be avoided where the drinking water has been fluoridated. In places where children are subjected to warm temperatures and consequently drink large amounts of water, a lower concentration of fluorine may be necessary to avoid mottling).

All the organizations of National Health in United States recommend the advantages and security of fluoridation. Among those organizations are: American Medical Association, American Dental Association, American Association of Public Health Dentists, American Public Health Association, State and Territorial Health Officers Association, United States Public Health Service, National Research Council, American Hospital Association, American Nurses' Association, American Water Works Association.

The fluoridation of water supplies has now been adopted by more than 150 cities. On the other hand, large centres, such as Philadelphia and maybe Cleveland, have adopted the project, and others such as New York, Chicago, etc., are contemplating the project but have not to date instituted this technique.

In Montreal, the question of fluoridation of the water supplied by the Municipal Water Works has already been laid before the Board of Health and the City Department of Health which are making a complete study thereof.

Therefore, from the medical standpoint to which I have limited my remarks, there is no objection in the fluoridation of water in the above-mentioned proportion, that is to say 1 ppm, under constant and rigid control. But, before giving a definite and complete decision, I consider it advisable to wait for the conclusions of the report actually under study by our Department Officials.

As Chief Medical Health Officer, I have not only to determine the efficiency and safety of the techniques, but also to consider other inherent problems such as practicability, cost and methods of control which concern especially the engineers.

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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS

OF THE

EIGHTEENTH MEETING

OF THE

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

OTTAWA

22 OCTOBER 1953

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Proceedings

of the

Eighteenth Meeting

of the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in the Board Room of the Faculty of Medicine, University of Montreal, 9:30 a.m., 22 October, 1953.

1. Attendance

Dr. J. Aldous
Dr. J.S. Bagnall
Dr. J.M. Beveridge
Dr. E. Charron
Dr. H. Copp
Dr. A.W. Ham
Dr. C.D. Husband
Dr. J.B. Macdonald
Dr. R.H. McDougall
Dr. E. Pagé
Dr. A.G. Racey
Brig. E.M. Wansbrough

Mr. S.J. Cook, Secretary

By invitation

Dr. C.H. Best -
Dr. A.H. Craven -
Dr. H.A. Hunter -
Dr. G. Nikiforuk -
Dr. K.J. Paynter -
Dr. C.P. Leblond -

Apologies for their absences were received from:

Dr. R.G. Ellis
Dr. H.K. Brown
Dr. A.C. Burton
Dr. J.D. Hamilton
Dr. L.A. Kilburn

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2. Election of Chairman

THE SECRETARY read a letter from Dr. R.G. Ellis, Chairman of the Committee, regretting that as he was confined to bed with a severe cold it would be impossible for him to be present at this meeting.

It was MOVED by Dr. Ham and SECONDED by Dr. Macdonald

That Dean E. Charron be invited to take the Chair for this meeting.

DEAN CHARRON thanked the members for having offered him the honour of presiding, but said that it would be necessary for him to leave the meeting at intervals during the day because of university business and that in the late afternoon a meeting of the Senate would take his full time. He begged therefore to be excused.

It was MOVED by Dr. McDougall and SECONDED by Dr. Ham

That Dr. J.B. Macdonald be asked to act as Chairman of this meeting. CARRIED.

3. Chairman's Remarks

THE CHAIRMAN said that he appreciated very highly indeed the honour of being chosen as acting chairman although he had hoped that the honour would be bestowed on someone else. He very much regretted that illness made it impossible for Dr. Ellis to be present and he was sure he spoke for the Committee in expressing the hope that Dr. Ellis would soon be fully recovered from his illness.

4. Minutes of the Seventeenth Meeting

THE CHAIRMAN said that he had one minor correction to suggest. In Min. 10, DR 35, he had been quoted as saying "that the report was entirely unexciting and entirely negative". He said he had intended to convey the impression that the results were "preliminary but necessary", and he asked that this correction be made.

It was MOVED by Dr. Charron and SECONDED by Dr. Bagnall

That the Minutes of the Seventeenth Meeting be corrected as noted above and that the Minutes be then taken as read and approved. CARRIED.

5. Business Arising Out of the Minutes

(i) Grant to Dr. L.F. Bélanger (Min. 4(i) 17th Mtg.)

A grant of $1340 to Dr. L.F. Bélanger for work on "Mechanism of bone and tooth formation in the light of autoradiography and histochemistry" was authorized by Letter Ballot of 2 April, 1953.

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It was MOVED by Dr. Charron and SECONDED by Dr. Bagnall

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A grant of $1340 to Dr. L.P. Bélanger for work on "Mechanism of bone and tooth formation in the light of autoradiography and histochemistry" was authorized by Letter Ballot of 2 April, 1953.

THE SECRETARY reported that he had received a letter dated 30 September, 1953, from Dr. Bélanger, advising him that Mr. George Ling who had been employed as a technician at $92 per month had worked only three months and had now left to take up a new position in Montreal. Dr. Bélanger asked whether he might use some or all of the $824 remaining in his salary appropriation for the purchase of an explosion-proof centrifuge which would cost him $845. He pointed out that he had received from the Medical Committee $250 towards the purchase of the centrifuge so that he really needed only about $600 or $650 from the Dental Committee.

DR. HAM thought that further information should be secured from Dr. Bélanger as to the need for the purchase of an explosion-proof centrifuge. He suggested, and it was AGREED that action in respect to Dr. Bélanger's request for the transfer of funds from salary to equipment for the purchase of an explosion-proof centrifuge be referred to the Executive for consideration and necessary action.

(ii) Dr. Bagnall's Bibliography (Min. 4(iii) 17th Mtg.)

THE SECRETARY reported that 216 copies had been sold and 40 complimentary copies distributed making a total of 256 to October, 1953. There remained on hand 64 copies at this date. Since the last meeting 24 additional copies had been distributed including 21 sold. It appeared therefore that the present rate of distribution was quite satisfactory and that the supply of the Bibliography was adequate at least for the present.

(iii) Reports (Min. 21, 17th Mtg.)

In all reports from grantees presented at this meeting the name of the institution and department in which the grantee works has been shown in each case in addition to the name of the grantee, as requested by the Committee.

As requested, also, arrangements have been made at this meeting for the presentation of project reports by special reviewers when the grantees are unable to be present to read their own reports.

(iv) Fellowship plan (Min. 24, 17th Mtg.)

THE SECRETARY reported that the scale of fellowship awards proposed by the Committee had been approved by the National Research Council at its March 1953 meeting, (Min. 30, 184th Mtg. NRC).

(v) Grant to Dr. C.B. Weld (Min. 28(a), 17th Mtg.)

THE SECRETARY reported that, in accordance with the wishes of the Committee, the grantee had been asked to re-submit his application expanding the project sufficiently to warrant the amount of the grant requested, and that his revised application had been referred to the Executive. It had been AGREED to make

an award of $2985 (instead of $4760). This provided $500 for animals and $2485 for salaries, being 7/12 of $4260 requested for the full year.

THE SECRETARY reported that, in accordance with the Committee's request, Dr. Nutlay had prepared a memorandum containing further information on the proposed project together with references. This had been sent to the Executive together with a revised outline of the proposed research prepared by Dr. Weld reading as follows:

> The objective of this research is to identify the histiocytes in the dental pulp of the rat incisor through their phagocytic nature. We shall refer to them in the course of our investigation as macrophages, thus placing emphasis on function (phagocytosis) and not origin (histiocytes). Since the pulp (of mesenchymal origin) of the rat incisor with its continuous growth, development and degeneration offers a suitable medium for the study of phagocytosis of the reticulo-endothelial system, the correct identification of its macrophages gains a distinctive interest. We shall attempt to determine their characteristics and position in the pulp of the rat incisor in relationship to other structural elements of that organ. We have failed to find any report of histologic investigation which has dealt with the identification of macrophages, scattered among the fibroblasts in the pulp.
>
> We shall first apply the technique of vital staining with the thought that the structural and functional character of the macrophages may be investigated with a fair amount of accuracy with regard to their identification. Then, post mortem staining methods would be used, using the various Agure-Eosin staining techniques. This will allow an evaluation of the results of vital and fixed tissue methods of staining, and a comparative study of the histochemical nature of the pulp macrophages. Finally, when these methods have given us our basic information on the macrophage, they will be applied to experimental material and the macrophage activity in inflammatory conditions, and after traumatic injury during healing and repair.
>
> The work will be done under the general supervision of the applicant in the Department of Physiology at Dalhousie University and the animals and tissues will be obtained by co-operation with the Department of Pathology of Camp Hill Hospital. The applicant has had considerable experience with histological investigations of several types and the associate who will be employed on the project, Dr. A.G. Nutlay (M.D., D.D.S.), is an interested and competent

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researcher who is experienced in this particular field. He is in practice and will devote about half his time to the project. A full time histological technician will also be needed. Full general equipment is available, money being needed only for expendable supplies - animals, chemicals, glassware, photographic material, etc.

THE SECRETARY pointed out also that Dr. Ellis had written to him, 8 September, 1953, saying that he wished to "re-emphasize that we must write to Dr. Weld soon after the October meeting and advise him that if an application for a renewal is to be submitted in March, either he or Dr. Nutlay should be prepared to attend the March meeting and advise the Committee of their plans and hopes for the project."

DR. CHARRON said he had been much impressed by Dr. Nutlay's presentation before the Committee and he would like to support and encourage him in his research. He thought it would be desirable, as had been suggested, to review the whole subject with Dr. Nutlay at the next meeting of the Committee in March, 1954.

THE CHAIRMAN reminded the members that as noted in the Proceedings of the Seventeenth Meeting (para. 1, p. 21) the grantee had been advised "that it was not the policy of the Committee to support senior scientific personnel under grants". Perhaps it would have been preferable to have made a grant directly to Dr. Nutlay instead of providing for his employment as a research worker under Dr. Weld's grant.

DR. HAM suggested that the Committee might reconsider this item of policy. He pointed out that the grantee has tried very hard to get a big piece of research started and he thought it was the duty of the Committee to encourage such efforts.

DR. BEST speaking as a past member of the Committee, and a past member of the National Research Council, said he was not aware that it was the policy of Council to oppose the making of grants when a senior scientist was to be employed to carry on the proposed research. He said that part-time experience with medical people frequently was unsatisfactory but in other cases brilliant results had been achieved by this means.

He added that, at the Banting and Best Department of Medical Research, many of the senior workers were dependent for their maintenance on medical research grants from various sources.

Moreover, many good grants had been made by the Medical Committee to persons who had been superannuated from other positions and this policy was resulting in much valuable work being done. He thought it was important to consider the individual scientist in each case and to act accordingly.

DR. HAM thought that a graduate in practice might like to do research but would need to have some financial assistance in order to pursue his problem on a satisfactory basis.

THE CHAIRMAN said the Committee should be very clear as to its policy in this matter.

(vi) Dr. M. Doreen Smith DR 13 (Min. 28, 17th Mtg.)

An application from Dr. Smith for a grant to enable her to study the fluoride content of dentine, enamel, and alveolar processes of teeth from Sarnia, Brantford, and Stratford, was received following the last meeting of the Committee and submitted by Letter Ballot to the Executive. As a result, an award of $1625 under DR 13 had been made to Dr. Smith.

(vii) Travel Grant to Dr. E.E. Johns (Min. 28, 17th Mtg.)

THE SECRETARY reported that as suggested in Min. 28(c), 17th Mtg. Dr. Johns had applied for a travel advance in order that he might visit one museum at the University of Pennsylvania.

Dr. Johns had reported to Dr. R.G. Ellis regarding this visit as follows:

Report on Visit to Philadelphia
by Dr. E.E. Johns

The material examined was drawn from three collections located in Philadelphia; the collection from the Wistar Institute, the Morton collection from the Academy of Natural Sciences and the Tepe Hissar Collection from the University of Pennsylvania Museum. Representative specimens were photographed from a total of over 3,700 skulls and detailed descriptions of the following were recorded.

(a) Amount of occlusal and incisal attrition. This was graded as O, A, B and C according to the extent of the wear.

(b) The relationship of the maxillary and mandibular molars and incisors.

(c) Cranial configuration with regard to gross asymmetries.

(d) A description of the diet utilized by the ethnic group examined.

(e) Approximate age and wherever possible sex of the individual.

(f) Pathology in the osseous and dental tissues.

The foregoing was accomplished through the co-operation of the curators of the collections and also the Department of Physical Anthropology, University of Pennsylvania.

The photographs include views of the occlusal surfaces of the teeth and also lateral and frontal views of the articulated maxillae. Where gross cranial asymmetries existed an attempt was made wherever possible to indicate this in the photographs.

A concentrated effort was made to secure specimens of deciduous and mixed dentitions and where any evidence of dental caries or periodontal lesions existed it was carefully recorded.

The following ethnic groups were represented in the material examined.

Indians

North American Indians
Tribes: Apache
Pueblo
Clickitak
Iroquois
Mound
Cliff Dwellers

Central American Indians
Tribes: Maya
Yucatan

South American Indians
Tribes: Auracanian
Toltecan (Peru)
Pachacamac (Peru)
Cachilaya
Aymans

(Native) African: Negro
Hottentots
Kraanian
South Liberian

U.S. Born

Negroes blended with Egyptians

Australian (New Holland)

Caucasians

Iranians (Tepe Hissar)
Swedish
English

Eskimoes (Alaska)

The foregoing was accomplished through the co-operation of the curators of the collections and also the Department of Physical Anthropology, University of Pennsylvania.

The photographs include views of the occlusal surfaces of the teeth and also lateral and frontal views of the articulated maxillae. Where gross cranial asymmetries existed an attempt was made wherever possible to indicate this in the photographs.

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Tribes: Apache
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Central American Indians
Tribes: Maya
Yucatan

South American Indians
Tribes: Aureanian
Toltecan (Peru)
Pachacamac (Peru)
Cachilaya
Aymara

(Native) African: Negro
Hottentots
Kreanian
South Liberian

U.S. Born

Negroes blended with Egyptians

Australian (New Holland)

Caucasians

Iranians (Tepe Hissar)
Swedish
English

Eskimoes (Alaska)

Chinese

Polynesians Sandwich Islands

It was suggested by the anthropologists consulted, Drs. Roberts, Farris, Rainey and Kidder, that it would be well worthwhile to see the Hooton Collections at Boston and the collections in New York. It was amazing to note the difference in the occlusion of those of the skulls which were from a group who did not cultivate cereals but were more dependent on a meat-type of diet.

THE CHAIRMAN pointed out that Dr. Johns wished to visit various other centres as part of his program. The Chairman said he would like the advice of the Committee as to its wishes in this matter.

After some discussion it was MOVED by Dr. McDougall and SECONDED by Dr. Bagnall

That the report by Dr. E.E. Johns on his visit to the University of Pennsylvania indicates that satisfactory progress is being made on this project. The Committee therefore recommends that the Chairman be authorized to make further travel grants to Dr. Johns for travel projects of a similar nature at his discretion. CARRIED.

(viii) Membership of the Committee (Min. 32, 17th Mtg.)

THE SECRETARY reported that the nominations for new members of the Committee made at the last meeting had been accepted and approved by the National Research Council (Min. 28, 184th Mtg. NRC)

The new members are: Drs. Aldous, Beveridge, Copp, Husband, and Racey.

Consideration of Reports from Grantees

At the meeting, the reports from grantees which were to be presented by guests were heard first, and afterwards the other reports from grantees were read, but for convenience of reference in the proceedings, the reports are arranged in numerical order of projects.

6. DR 1 - Dr. J.J. Rae
Reported by Dr. G. Nikiforuk

"Chemical mechanisms in dental caries"

The summary appears in APPENDIX "I"

DR. NIKIFORUK read the summary and the following paragraph from a letter written to the Secretary by Dr. Rae, 13 October, 1953:

"The situation in regard to DR 1 is that Miss Shemilt left at the middle of September and there are one or two wind-up experiments I would like to see done before I write a definitive report. I have not been able to do these experiments as yet, and would like to ask the Committee to accept the summary I submitted to you as an interim report. I hope to complete the ammonia project within the next few weeks."

DR. NIKIFORUK said that he understood this was the final report on the ammonia work, but that Dr. Rae hoped to continue his investigational work on enamel.

7. DR 13 - Dr. M. Doreen Smith
Reported by Dr. Page

"Fluoride content of dentine, enamel and alveolar processes of teeth for Sarnia, Brantford and Stratford"

The report appears in APPENDIX "M"

DR. PAGE pointed out that the results of this study are summarized in Table 3, page "M-4" from which it was apparent that fluoridation of the Brantford water supply had led to an increase in the percentage of fluorine in both dentine and enamel of Brantford children's teeth, comparable with the results observed in the teeth of Stratford children. He suggested that as the Department of National Health and Welfare was conducting municipal surveys to determine the effect of fluoridation of municipal water supplies on teeth, the work being done by Dr. Doreen Smith might be supported from funds provided under the Health grants rather than from Dental Committee funds.

DR. NIKIFORUK said that so far as he knew, no other work was being done on artificially treated water sources.

DR. HAM suggested that the Department of National Health and Welfare might approach Dr. Smith with a view to supporting her work out of their appropriation.

In the discussion which followed it was agreed that no action need be taken by the Committee in this matter at the present time.

8. DR 22 - Dr. C.H. Best
Reported by Dr. Best and Dr. A.W. Ham

"An investigation of the mechanism of repair of the supporting structures of the teeth"

The summary and report appear in APPENDIX "O"

DR. BEST reviewed the summary and report briefly.

"The situation in regard to DR 1 is that Miss Shenkit left at the middle of September and there are one or two wind-up experiments I would like to see done before I write a definitive report. I have not been able to do these experiments as yet, and would like to ask the Committee to accept the summary I submitted to you as an interim report. I hope to complete the ammonia project within the next few weeks."

DR. NIKIFORUK said that he understood this was the final report on the ammonia work, but that Dr. Rae hoped to continue his investigational work on enamel.

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DR. PAGE pointed out that the results of this study are summarized in Table 3, page "M-4" from which it was apparent that fluoridation of the Brantford water supply had led to an increase in the percentage of fluorine in both dentine and enamel of Brantford children's teeth, comparable with the results observed in the teeth of Stratford children. He suggested that as the Department of National Health and Welfare was conducting municipal surveys to determine the effect of fluoridation of municipal water supplies on teeth, the work being done by Dr. Doreen Smith might be supported from funds provided under the Health Grants rather than from Dental Committee funds.

DR. NIKIFORUK said that so far as he knew, no other work was being done on artificially treated water sources.

DR. HAM suggested that the Department of National Health and Welfare might approach Dr. Smith with a view to supporting her work out of their appropriation.

In the discussion which followed it was agreed that no action need be taken by the Committee in this matter at the present time.

8. DR 22 - Dr. G.H. Best
Reported by Dr. Best and Dr. A.W. Ham

"An investigation of the mechanism of repair of the supporting structures of the teeth"

The summary and report appear in APPENDIX "O"

DR. BEST reviewed the summary and report briefly.

DR. HAM who had been asked to study the report on DR 22 presented the following written comment.

"As you all know, in serious periodontal disease the periodontal membrane becomes detached from the side of the tooth and atrophies. This results in a pocket being formed, a pocket which is bounded on one side by the naked tooth and on the other by the detached tissue. Commonly this condition is associated with some degree of resorption of the alveolar bone, and with epithelium growing down from the gingiva to line the tissue which has become detached from the tooth. Commonly, also, the tissue that constitutes the outer wall of the pocket, evidences varying degrees of inflammatory cell infiltration.

"Dr. Linghorne has sought to produce experimental pockets in dogs to determine whether or not alveolar bone can be regenerated and also to determine whether or not new tissue will form in the region of the periodontal membrane which will both fill in the pocket and become firmly attached to the tooth. It is obvious that an experimental approach to this subject has the obvious advantage of permitting tissue to be obtained, sectioned, and studied under the microscope, so that definite evidence regarding whether or not these phenomena occur can be obtained.

"Over the past few years, in experiments which are necessarily long drawn out and time-consuming, Dr. Linghorne has shown that after an artificial pocket has been created, new tissue will develop in the periodontal membrane and that this tissue will cause cementum to be formed on the naked dentine so that the new tissue in the periodontal membrane becomes firmly attached to the tooth. Dr. Linghorne has shown, moreover, in histological section, that at least a portion of the alveolar process which has been removed will be regenerated. He has, therefore, provided definite evidence of reparative capacities in this part of the body. (2 publications)

"Dr. Linghorne, in these studies, has investigated the origin and differentiation of the new tissue that forms to repair the pocket and this has involved his becoming involved in several fundamental problems about bone and connective tissue regeneration. Dr. Linghorne, in my opinion, is becoming increasingly well versed on matters pertaining to these controversies and is contributing to their solution.

"Dr. Linghorne is aware of the fact that the experimental pockets he creates in dogs are not precisely the same as the pockets that occur in man

DR. MAM who had been asked to study the report on DR 22 presented the following written comment.

"As you all know, in serious periodontal disease the periodontal membrane becomes detached from the side of the tooth and atrophies. This results in a pocket being formed, a pocket which is bounded on one side by the naked tooth and on the other by the detached tissue. Commonly this condition is associated with some degree of resorption of the alveolar bone, and with epithelium growing down from the gingiva to line the tissue which has become detached from the tooth. Commonly, also, the tissue that constitutes the outer wall of the pocket, evidences varying degrees of inflammatory cell infiltration.

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"Dr. Lindhorne is aware of the fact that the experimental pockets he creates in dogs are not precisely the same as the pockets that occur in man

as a result of disease. Recently, therefore, he has been attempting to modify his experimental procedures to attempt to make a pocket which is more comparable to that which develops in man. He has devised a method which permits the artificial pocket to become lined with epithelium, and which also permits any periosteum left in the detached tissue to atrophy. His study on the sequence of events that occur in these pockets is under way and he has only preliminary results to date and intends to report further on this matter at the Spring meeting of the Committee. So far, however, he has shown that although the process is slower, both attachment and alveolar bone regeneration occur under these conditions (one publication, in press). He has recently, however, altered his procedure in that healing may be both more direct and more rapid.

"Along with his main study Dr. Linghorne has been studying the periodontal structures under conditions of different hormone imbalances; material for this is available from other experiments being conducted in Dr. Best's Department. To date he has made an interesting observation that I interpret to mean that the collagen fibers that form under conditions of hypophysectomy have a different staining reaction from those that form in a normal animal; they stain like the fibroglia fibers that Mallory described years ago; with the P.T.A. technique they stain similarly to fibrin. It is possible that this may be of some significance with regard to the problem of how different hormones affect the formation of intercellular substances.

"It is my feeling that Dr. Linghorne is making steady progress in a line of work in which experimental work is both difficult and necessary and I should greatly regret his losing the opportunity to contribute further in this field".

THE CHAIRMAN drew the attention of the Committee to comments on this project which appeared in the Proceedings of the Seventeenth Meeting. He noted that in Min. 8 it had been observed that "this project may have reached the stage where Linghorne should be regarded as a senior researcher in this field and that the Committee could not continue to support him as such indefinitely."

And again, in Min. 28, when the grant was under consideration the following comment was made:

"The Committee was of the opinion that it is not desirable to make substantial contributions to the income of relatively senior scientific workers for more than a limited number of years for the obvious reason that such workers may come to depend considerably

on this income whereas the granting body is by its terms of reference required to reconsider its commitments annually and has no certain continuance. A letter in this sense is to be sent to the grantee with a view of terminating Dr. Linghorne's appointment at the end of the coming year".

DR. BAGNALL said this appeared to be a long-term project on which good results were being obtained. He thought the work should be reviewed at regular intervals but he saw no objection to continuing the project.

DR. COPP inquired whether a senior research fellowship might not be awarded to Dr. Linghorne for the conduct of this investigation.

THE CHAIRMAN said that all members seemed to be agreed that project DR 22 represented good work which merited the support of the Committee. The only question involved concerned the method by which Dr. Linghorne is to be supported while doing this work. If provision could be made under a senior dental fellowship for the part-time employment of Dr. Linghorne, it might be possible to carry the fellowship forward for a longer period than one year at a time which is all that can be done under a Dental Committee grant. There was, of course, no application under consideration at this time and he was permitting the discussion on this subject merely because Dr. Best was present and able to answer questions put to him by the members.

DR. BEVERIDGE suggested that Dr. Best and Dr. Linghorne look into the possibility of applying for a fellowship rather than a grant and make their submission to the Committee accordingly.

DR. BEST said that in the Banting and Best Department of Medical Research it was not unusual for senior staff to be supported entirely on funds derived from grants-in-aid and, speaking as a former member of the National Research Council, he saw no objection to a continuance of the present arrangements for the support of Dr. Linghorne's work. He pointed out that Dr. Linghorne was most scrupulous in giving half of his time to this research. Dr. Best said that he would prefer to have the project listed in Dr. Linghorne's name but he recognized that if this were done Dr. Linghorne would be unable to draw any financial support for himself from the Committee funds. Accordingly, Dr. Best said he proposed to file an application for a Senior Research Fellowship for Dr. Linghorne at the next meeting of the Committee and, if this application failed, he would seek to have the work supported in some other way.

9. DR 23 - Dr. C.P. Leblond
Reported by Dr. Leblond

"Entry of phosphate, carbonate and calcium into teeth, as shown with the help of radioactive elements"

A summary and report by the grantee and a report by Miss Yurika Kumamoto appear in APPENDIX "K"

on this income whereas the granting body is by its terms of reference required to reconsider its commitments annually and has no certain continuance. A letter in this sense is to be sent to the grantee with a view of terminating Dr. Lingborne's appointment at the end of the coming year".

DR. BAGNALL said this appeared to be a long-term project on which good results were being obtained. He thought the work should be reviewed at regular intervals but he saw no objection to continuing the project.

DR. COPP inquired whether a senior research fellowship might not be awarded to Dr. Lingborne for the conduct of this investigation.

THE CHAIRMAN said that all members seemed to be agreed that project DR 22 represented good work which merited the support of the Committee. The only question involved concerned the method by which Dr. Lingborne is to be supported while doing this work. If provision could be made under a senior dental fellowship for the part-time employment of Dr. Lingborne, it might be possible to carry the fellowship forward for a longer period than one year at a time which is all that can be done under a Dental Committee grant. There was, of course, no application under consideration at this time and he was permitting the discussion on this subject merely because Dr. Best was present and able to answer questions put to him by the members.

DR. BEVERIDGE suggested that Dr. Best and Dr. Lingborne look into the possibility of applying for a fellowship rather than a grant and make their submission to the Committee accordingly.

DR. BEST said that in the Banting and Best Department of Medical Research it was not unusual for senior staff to be supported entirely on funds derived from grants-in-aid and, speaking as a former member of the National Research Council, he saw no objection to a continuance of the present arrangements for the support of Dr. Lingborne's work. He pointed out that Dr. Lingborne was most scrupulous in giving half of his time to this research. Dr. Best said that he would prefer to have the project listed in Dr. Lingborne's name but be recognized that if this were done Dr. Lingborne would be unable to draw any financial support for himself from the Committee funds. Accordingly, Dr. Best said he proposed to file an application for a Senior Research Fellowship for Dr. Lingborne at the next meeting of the Committee and, if this application failed, he would seek to have the work supported in some other way.

9. DR 23 - Dr. G.P. Lebland
Reported by Dr. Lebland

"Entry of phosphate, carbonate and calcium into teeth, as shown with the help of radioactive elements"

A summary and report by the grantee and a report by Miss Yuriko Kumamoto appear in APPENDIX "K"

DR. LEBLOND presented the summary and the two reports in detail, giving a clear and concise review of the work.

On the conclusion of his remarks, Dr. Leblond was very highly complimented by the Chairman, Dr. Copp, Dr. Best, and Dr. Ham, on the brilliant research which he had done. It was the opinion of the members that this was an excellent piece of fundamental work.

DR. LEBLOND thanked the members and said that radioautography was yielding valuable information to histologists.

10. DR 28 - Dr. O.J. Walker
Reported by Dr. C.D. Husband

"Investigation of methods for destroying the organic matter in teeth, bones and other materials prior to determination of fluorine content"

The summary and report appear in APPENDIX "L"

DR. HUSBAND presented both the summary and the report on this project.

DR. BEVERIDGE inquired whether the grantee was having difficulty with the method of analysis. He seemed to be securing consistently high blanks. No correction for the high blanks was apparent in assessing the accuracy of the technique.

DR. COPP thought it might be useful to have another chemist examine the data and report to the Committee.

DR. HAM suggested that Dr. Beveridge have prepared by a chemist a memorandum on this report which could be sent to Dr. Walker by the Secretary of the Committee.

It was AGREED that this should be done. (See note in Appendix "L")

11. DR 35 - Dr. J.B. Macdonald
Reported by Dr. Macdonald

"Experimental fusospirochetal infection"

The summary and report appear in APPENDIX "J"

DR. MACDONALD briefly reviewed the development of this project. In regard to the report he had submitted, he asked that a correction be made by deleting item "IV-Change in virulence of unpurified guinea pig exudate in relation to repeated passage", from page "J-5". He said that the evidence in Table 5 was insufficient, he now thought, to warrant the remarks on change in virulence which he had previously made. For the same reason he also wished to have item 4 deleted from the summary.

THE SECRETARY said this change would be made in APPENDIX "J".

DR. LEDBLOND presented the summary and the two reports in detail, giving a clear and concise review of the work.

On the conclusion of his remarks, Dr. Ledblond was very highly complimented by the Chairman, Dr. Copp, Dr. Best, and Dr. Ham, on the brilliant research which he had done. It was the opinion of the members that this was an excellent piece of fundamental work.

DR. LEDBLOND thanked the members and said that radioautography was yielding valuable information to histologists.

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DR. COPP thought it might be useful to have another chemist examine the data and report to the Committee.

DR. HAM suggested that Dr. Beveridge have prepared by a chemist a memorandum on this report which could be sent to Dr. Walker by the Secretary of the Committee.

It was AGREED that this should be done. (See note in Appendix "L"

11. DR 35 - Dr. J.B. Macdonald
Reported by Dr. Macdonald

"Experimental Tuscaprotchetal Infection"

The summary and report appear in APPENDIX "J"

DR. MACDONALD briefly reviewed the development of this project. In regard to the report he had submitted, he asked that a correction be made by deleting item "IV-Change in virulence of unpurified guinea pig exudate in relation to repeated passage", from page "J-5". He said that the evidence in Table 5 was insufficient, he now thought, to warrant the remarks on change in virulence which he had previously made. For the same reason he also wished to have item 4 deleted from the summary.

THE SECRETARY said this change would be made in APPENDIX "J".

DR. HAM inquired whether cortisone should be tried.

DR. MACDONALD said he had this in mind.

12. DR 37 - Dr. H. Jenkins
Reported by Dr. J.B. Macdonald

"Further studies in the electromyography of the head and neck musculature"

The summary appears in APPENDIX "S"

DR. MACDONALD outlined the work that was being done under this investigation and at his suggestion it was AGREED to invite Dr. H. Jenkins to report in person on this project at the March meeting.

13. DR 38 - Dr. K.J. Paynter
Reported by Dr. A.W. Ham

"The effect of the endocrines on the teeth and jaws. II. The effect of propyl thiouracil on the bone of the jaws of rats"

The summary and report appear in APPENDIX "M"

DR. HAM said that this work had been started at Columbia University when Dr. Paynter was doing his Ph.D. work there. He read the summary and part of the report drawing attention particularly to the grantee's wish that the title be changed to read as follows: "The effect of the endocrines on the teeth and jaws. II. Further studies on the effect of thiouracil on the development of molar teeth of rats".

It was AGREED that this request for a change in the title of the project should be approved.

DR. COPP said he would like to look up some references and that he would send them in shortly for onward transmission to the grantee.

DR. BEST said that Dr. Becks had done a great deal of work on this subject.

14. DR 43 - Dr. S.G. Davis and Dr. H.R. MacLean
Reported by Dr. McDougall

"Electropotentials caused by dental alloys"

The summary and report appear in APPENDIX "F"

15. DR 44 - Dr. A.H. Craven
Reported by Dr. Craven

"The growth in width of the face and head of the Macaque monkey as revealed by vital staining"

The summary and report appear in APPENDIX "D"

DR. MAM inquired whether corticone should be tried.

DR. MACDONALD said he had this in mind.

12. DR 37 - Dr. H. Jenkins
Reported by Dr. J.B. Macdonald

"Further studies in the electromyography of the head and neck musculature"

The summary appears in APPENDIX "S"

DR. MACDONALD outlined the work that was being done under this investigation and at his suggestion it was AGREED to invite Dr. H. Jenkins to report in person on this project at the March meeting.

13. DR 38 - Dr. K.J. Paynter
Reported by Dr. A.W. Ham

"The effect of the endocrines on the teeth and jaws. II. The effect of propyl thiouracil on the bone of the jaws of rats"

The summary and report appear in APPENDIX "T"

DR. HAM said that this work had been started at Columbia University when Dr. Paynter was doing his Ph.D. work there. He read the summary and part of the report drawing attention particularly to the grantee's wish that the title be changed to read as follows: "The effect of the endocrines on the teeth and jaws. II. Further studies on the effect of thiouracil on the development of molar teeth of rats".

It was AGREED that this request for a change in the title of the project should be approved.

DR. COPP said he would like to look up some references and that he would send them in shortly for onward transmission to the grantee.

DR. BEST said that Dr. Becks had done a great deal of work on this subject.

14. DR 43 - Dr. S.G. Davis and Dr. N.R. Maclean
Reported by Dr. McDougall

"Electropotentials caused by dental alloys"

The summary and report appear in APPENDIX "U"

15. DR 44 - Dr. A.H. Craven
Reported by Dr. Craven

"The growth in width of the face and head of the Macaque monkey as revealed by vital staining"

The summary and report appear in APPENDIX "V"

DR. CRAVEN in presenting his report announced also that he had been appointed to a staff post at the University of New Zealand and would be leaving shortly to take up his new duties. He suggested therefore that further funds on this grant be held in abeyance.

16. DR 46 - Dr. H. Jenkins
Reported by Dr. J.B. Macdonald

"Study of treated cases"

Two summaries (i) Classification of records and (ii) An analysis of treated cases, appear in APPENDIX "T"

DR. MACDONALD said that the purpose of this investigation was to make a collection available for research use by graduate students; the second summary presented the first report based on a study of this collection. He noted that Dr. Moyers who was now at Ann Arbor had been replaced as grantee by Dr. Jenkins. A letter had been received from Dr. Moyers by the Secretary which contained the following paragraph:

"Would you be so kind as to convey to the members of the Committee my personal thanks and appreciation for the very generous manner in which they supported my work during my stay at the University of Toronto. I trust that the final reports will justify the faith they have displayed in our projects. It has been an extreme pleasure to know and work with you and the members of the Committee."

17. DR 47 - Dr. H.A. Hunter
Reported by Dr. Hunter

"The pathological characteristics of experimental fusospirochetal infections"

The summary and report appear in APPENDIX "B"

DR. HUNTER reviewed his work in some detail.

DR. HAM referring to the last paragraph on page "B-2" inquired whether Graham had used any controls to determine liver damage from chloroform. Dr. Hunter stated that this was not clear in Graham's report. He said the whole program seemed designed to find out whether infection in periodontal pockets affects other organs, and was actually a repetition of Dr. Graham's work.

THE CHAIRMAN said he thought that Dr. Graham had doubts concerning his results and had not been surprised that Dr. Hunter had obtained different results on repeating the experiment.

DR. COPP suggested that comparison be made of different methods of creating infection e.g., subgingivally and intravenously.

DR. BEVERIDGE asked whether there was any way of standardizing filtrates from pooled scrapings. It was not thought this could be done.

18. DR 48 - Dr. G. Nikiforuk
Reported by Dr. Nikiforuk

"The effect of topical application of silicones in protecting teeth against acid solutions in vitro"

The summary appears in APPENDIX "C"

DR. NIKIFORUK presented his material as a final report on silicones. He said that a general screening of silicone materials had been done and that it would now be possible for him to get along in this field without an outside grant. He was working with students and had ample facilities for his work in the Dental Faculty at Toronto.

DR. ALDOUS asked whether any physiological effects from the use of silicones had been observed.

DR. NIKIFORUK replied that the only case in which such effects were observed, occurred in the use of ethyl silicates which are hemolytic. He thought that publication of his results would undoubtedly lead to commercial exploitation of his findings.

THE CHAIRMAN and DR. BEST both pointed out that Canadian Patents and Development Limited, a Crown company set up under the National Research Council, would be in a position to take up Dr. Nikiforuk's findings and make them available to industry.

19. DR 49 - Dr. A.H. Craven
Reported by Dr. Craven

"Posture of the head in man. I. A recording device to determine postural changes of the head in the horizontal plane"

The summary appears in APPENDIX "E"

DR. NIKIFORUK, noting that difficulty was being encountered in coating the inside of the tube with resistance paint, suggested that as silicone resins have very good dielectric properties, they might be useful as resistance paint material for this purpose.

20. DR 50 - Dr. J.B. Macdonald
Reported by Dr. Macdonald

"The isolation and characteristics of Bacteroides nigrescens"

The summary appears in APPENDIX "G"

DR. MACDONALD said that he proposed to seek the growth factor as much good background material was available for study. The work was interesting but the project was in a very early stage.

21. DR 51 - Dr. R.L.de C.H. Saunders
Reported by Dr. Bagnall

"Study of human dental pulp blood vessels by X-ray micro-arteriographic methods"

Summary appears in APPENDIX "A"

DR. BAGNALL presented the grantee's summary and read the following note from him which contains some further particulars regarding the project.

"At present transverse sections of teeth (all available) are being started, since hitherto only longitudinal studies have been made.

"The histological preparations already completed are being studied microscopically in order to provide a background for the interpretation of the microradiography of such vessels.

"Since the report was made more satisfactory microradiography results have been secured by using a mercury vapour sensitised emulsion permitting the visualisation of capillaries in other test tissues.

"Just now we are substituting a special injectant for that previously used, which we now know readily enters capillaries and is capable of throwing a satisfactory picture of the capillary bed when microradiographed. This material we have chosen is micropaque, which is a stabilised micro-dispersion of barium sulphate with an average particle size of below 0.5 microns. We are optimistic that it may prove useful both radiographically and histologically for our studies."

It was AGREED that Dr. Saunders should be invited to attend the next meeting of the Committee to report in person on his project.

22. DR 52 - Dr. L.F. Bélanger
Reported by Dr. C.P. Leblond

"Mechanism of bone and tooth formation in the light of autoradiography and histochemistry"

The summary and reprint appear in APPENDIX "H"

DR. LEBLOND briefly discussed Dr. Bélanger's work.

23. DR 53 - Dr. C.B. Weld

"The macrophages in the pulp tissue of the rat incisor and lower jaw"

No report on this project was received as the grant was only made in mid-September, 1953.

24. Invitation to Grantees

THE CHAIRMAN stressed the importance and value of the Committee policy whereby grantees are invited to be present from time to time to report on their projects. He noted that at the present meeting it had been suggested that invitations be issued to grantees to be present at the next meeting of the Committee as follows: Dr. L.F. Bélanger, Dr. H. Jenkins, Dr. A. Nutlay, (Weld) and Dr. R.L.de C.H. Saunders. + Langhorne

25. Fellowships (Min. 23, 17th Mtg.)

(i) Application from Dr. Clifford Reynolds

THE SECRETARY presented an application from Dr. Clifford Reynolds 241 Poyntz Avenue, Willowdale, Ont., who is proceeding to the Master of Science degree in the Faculty of Dentistry, University of Toronto. He pointed out that the application was incomplete in some details, and that Dr. Reynolds had passed the prescribed age limit and that his application had not been made within the time specified under the regulations. These facts had been pointed out to the applicant, but at the suggestion of Dr. Ellis, the application was now brought to the attention of the Committee as it was the only fellowship application received.

Considerable discussion ensued in which it was AGREED to tell Dr. Reynolds that his application was late and that it did not contain all of the information called for and that therefore no action could be taken by the Committee at this time.

In the discussion it was pointed out that, as a graduate student in his first year, Dr. Reynolds would have little or no time for research and would therefore be unable to carry out the duties expected of a fellowship holder. On the other hand it seemed possible that he was looking forward a year and had filed this application as a preliminary move to secure financial assistance later on.

It was MOVED by Dr. Copp and SECONDED by Dr. Bagnall

That the Committee take no action on the application of Dr. Reynolds for a fellowship at this time, but that the applicant be advised that consideration might be given at the March meeting if a fully documented application were in the hands of the Secretary prior to the next closing date for the receipt of applications. CARRIED.

23. DR 53 - Dr. G.B. Weld

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In the discussion it was pointed out that, as a graduate student in his first year, Dr. Reynolds would have little or no time for research and would therefore be unable to carry out the duties expected of a fellowship holder. On the other hand it seemed possible that he was looking forward a year and had filed this application as a preliminary move to secure financial assistance later on.

It was MOVED by Dr. Copp and SECONDED by Dr. Baynall

That the Committee take no action on the application of Dr. Reynolds for a fellowship at this time, but that the applicant be advised that consideration might be given at the March meeting if a fully documented application were in the hands of the Secretary prior to the next closing date for the receipt of applications. CARRIED.

26. Fellowship Plans (Min. 24, 17th Mtg.)

DR. HAM suggested that the application forms used for fellowships might be revised to provide additional information. He thought there should be an item to show what each fellowship applicant proposes to do in research and that provision should be made to have all fellowship applications reviewed before they are presented to the Committee. He proposed that item 19 of the application form be enlarged to provide for an outline of research which he thought should be prepared in collaboration with the supervisor and should give details of the project and the methods to be used.

DR. COPP agreed with Dr. Ham, but said that it was desirable to retain flexibility. He thought it should be sufficient to know the applicant's general plans without the details.

DR. BEVERIDGE was also of the opinion that the nature of the problem proposed should be sufficient.

THE CHAIRMAN said that the objective of the fellowship was to give a man training in basic sciences and experience in research and that in most instances neither the applicant nor his adviser would be in a position to provide much information in respect to the research program at the beginning of the training periods.

After some further discussion it was MOVED by Dr. Ham and SECONDED by Dr. Copp

That the Executive be asked to review the fellowship application form and to report at the March meeting. CARRIED.

27. Application for Grant (Min. 28, 17th Mtg.)

THE SECRETARY reported that he had received an inquiry from Dr. B.N. Kropp, Laboratory of Histology and Embryology, Queen's University, Kingston, Ont., for particulars regarding the method of filing applications for grants under the Dental Committee. This information had been supplied.

In a letter dated 21 October, 1953, Dr. Kropp submitted an application in the amount of $820 for work on "Development of a rapid method for grinding thin sections of plastic embedded teeth".

The applicant submitted a paper describing work on grinding thin sections of plastic embedded bone and said that his proposed research was to enable him to modify and adapt this method for use with calcified teeth. The method has the advantage that the complete permanently mounted specimen can be prepared in an hour or less.

DR. HUNTER said the work of grinding sections was enormous and generally it was done by hand.

DR. HAM pointed out that Dr. Belanger has used a similar method.

DR. CRAVEN observed that the applicant stated he had been able to make sections as thin as ten microns, which he thought remarkable.

It was MOVED by Dr. Copp and SECONDED by Dr. Beveridge

That the application of Dr. B.N. Kropp for $820 for the "Development of a rapid method for grinding thin sections of plastic embedded teeth" be approved and that the applicant be advised that it will be necessary for him to file a new application if he wishes to continue working in this field in 1954-55. This project will be known as DR 54. CARRIED.

28. Finances (Min. 22, 17th Mtg.)

THE SECRETARY reported on the financial standing of the Dental Committee as at 30 September, 1953, as follows:

Allotment	$40,700	Grants awarded by Committee	$30,075
Authorized Carry-over from 1952-53 for Commitments	462.18	Authorized Carry-over from 1952-53 for Commitments	462.18
		Travel	235.72
		Contingencies	116.49
		Unallotted	10,272.79
	41,162.18		41,162.18

29. Appointment to the Executive (Min. 32, 7 Mtg.)

THE SECRETARY reported that no replacement had been made on the Executive following the retirement of Dr. D.L. Thomson. He suggested that the Committee might give consideration to this matter.

THE CHAIRMAN invited the members to submit nominations.

It was MOVED by Dr. McDougall and SECONDED by Dr. Beveridge

That Dr. A.W. Ham be appointed as a member of the Executive in succession to Dr. D.L. Thomson. CARRIED.

30. Special Travel Grants (Min. 30, 17th Mtg.)

THE CHAIRMAN said that Dr. Ellis had especially requested that consideration be given at this meeting to the authorization of special travel grants whereby grantees might be enabled to attend scientific meetings such as the International Association

for Dental Research. Last year provision had been made for seven such grants limited in each case to $150 and subject to the usual regulations covering travel appropriations.

It was pointed out in discussion that while provision had been made only to cover the attendance of grantees at the International Association for Dental Research, a further application had been received last year to permit the attendance of a grantee at the American Association of Anatomists' meeting.

It was MOVED by Dr. Copp and SECONDED by Dr. Aldous

(i) That the Associate Committee on Dental Research authorize the making of not more than seven special travel awards in 1954-55, each in an amount not to exceed $150, to enable grantees under the Committee to attend scientific meetings such as the International Association for Dental Research;

(ii) That each such award be made subject to the NRC requirements of accounting for travel funds; and,

(iii) That the awards be made at the discretion of the Executive. CARRIED.

31. Radiation Hazards

THE SECRETARY said that Dr. R.G. Ellis had received a memorandum from Dr. R.J. Godfrey, Professor of Prosthetic Dentistry, Toronto, Ont., raising a question in regard to the effects of atomic blasts on metal restorations in the mouths of humans. The memorandum is as follows:

The Effects of Atomic Blasts on Metal Restorations in the Mouths of Humans

Since the atomic bomb blasts in Japan during the last war, it is not known if any observations were made or reported on the effect of radioactivity on metal fillings or metal dentures in the mouths of those affected by the blasts.

With the advanced research in nuclear energy and the possible effects on human beings who may come into contact with radioactivity from this source, it would be valuable to know if radioactivity would be induced in fillings, inlays and partial dentures constructed in gold alloys and nickel-chrome alloys and worn in the mouth.

It has been reported by the Consolidated Mining and Smelting Company of Canada that indium, a metal used in dental alloys instead of zinc, is affected by radioactivity and that indium is used as an indicator in the atomic pile.

for Dental Research. Last year provision had been made for seven such grants limited in each case to $150 and subject to the usual regulations covering travel appropriations.

It was pointed out in discussion that while provision had been made only to cover the attendance of grantees at the International Association for Dental Research, a further application had been received last year to permit the attendance of a grantee at the American Association of Anatomists' meeting.

It was MOVED by Dr. Copp and SECONDED by Dr. Aldous

(i) That the Associate Committee on Dental Research authorize the making of not more than seven special travel awards in 1954-55, each in an amount not to exceed $150, to enable grantees under the Committee to attend scientific meetings such as the International Association for Dental Research;

(ii) That each such award be made subject to the NRC requirements of accounting for travel funds; and,

(iii) That the awards be made at the discretion of the Executive. CARRIED.

21. Radiation Hazards

THE SECRETARY said that Dr. R.G. Ellis had received a memorandum from Dr. R.J. Godfrey, Professor of Prosthetic Dentistry, Toronto, Ont., raising a question in regard to the effects of atomic blasts on metal restorations in the mouths of humans. The memorandum is as follows:

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With the advanced research in nuclear energy and the possible effects on human beings who may come into contact with radioactivity from this source, it would be valuable to know if radioactivity would be induced in fillings, inlays and partial dentures constructed in gold alloys and nickel-chrome alloys and worn in the mouth.

It has been reported by the Consolidated Mining and Smelting Company of Canada that indium, a metal used in dental alloys instead of zinc, is affected by radioactivity and that indium is used as an indicator in the atomic pile.

Although very small quantities of indium are used in dental alloys, it would be useful to know if this metal or other metals would produce a hazard to persons if they came into contact with atomic blasts.

It is felt that some investigation of this problem might well be undertaken.

THE SECRETARY said that he had consulted technical personnel in Atomic Energy of Canada and had been advised that although there have been references in the literature to the effect of radiation on teeth particularly in the young, it seems obvious that this is well understood. No references to the specific effect of radiation on fillings have been noticed.

The opinion was expressed by the consultant that in order to induce radioactivity in metallic tooth fillings it would be necessary for the person concerned to be exposed directly to a neutron beam (or very powerful gamma ray) and that in such circumstances the person would have received a lethal dose of neutrons before any of his metallic fillings became radioactive.

It was the expert's view that no problem exists in respect of radiation hazards in connection with metallic fillings. The calcium content of the teeth is in itself a reasonably good shield.

It was AGREED that the Secretary should send a letter to Dr. Godfrey in the sense of these remarks.

32. Appreciation

DR. BAGNALL said that before the Committee adjourned he would like on behalf of the members to express their appreciation to Dr. Charron for his kindness in making arrangements for the Committee to hold this meeting in the University of Montreal, which had been a great convenience, and to extend to the Vice-Rector, Monseigneur Deniger, the sincere thanks of all the members for the hospitality afforded the Committee by the University of Montreal, including the excellent lunch which had been provided in the Hospital Dining Room.

His remarks were received with applause.

DR. CHARRON said that the authorities of the University of Montreal were delighted to have the opportunity of receiving the Associate Committee on Dental Research and thus giving recognition to the great contributions made by the National Research Council to the support of the University's varied activities. The meeting had been an occasion for mutual gratification and he was very pleased to have had a small part in making the preliminary arrangements.

33. Date of Next Meeting (Min. 35, 17th Mtg.)

After a brief discussion it was AGREED that the date of the next meeting should be set by the Chairman and the Secretary with special regard to the date of the meeting of the Advisory Committee on Medical Research and any other meeting in which members of the Dental Committee were likely to be concerned.

The meeting adjourned at 4:40 p.m.

23. Date of Next Meeting (Min. 35, 17th Mtg.)

After a brief discussion it was AGREED that the date of the next meeting should be set by the Chairman and the Secretary with special regard to the date of the meeting of the Advisory Committee on Medical Research and any other meeting in which members of the Dental Committee were likely to be concerned.

The meeting adjourned at 4:40 p.m.

APPENDIX "A"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: Study of human dental pulp blood vessels by X-ray micro-arteriographic methods.

Grantee: Dr. R.L. de C.H. Saunders.

Project No.: DR 51 Amount of Grant: $1200

SUMMARY OF WORK

The Kramer technique of injecting the blood vessels of the dental pulp has been repeated a number of times using various injection masses such as India Ink and Sodium Citrate, Thorotrast (20%, 50%) both untinted and tinted with Red Speedball Ink, successive injections of Sodium Chloride and Silver Nitrate (aqueous 1%), and also Sodium Iodide (50%).

As a result of varying the injectants it has been found that sodium citrate solution weakly tinted with India Ink (5%) best demonstrated histologically the pulp blood vessels. This solution is weaker than that employed by Kramer (Anat. Rec. 91-100, 111, Sept. 1951).

Following standardization of the negative pressures and time factor required to satisfactorily inject the human pulp blood vessels, and the arrival at the best injection mass for these vessels, the teeth were studied histologically to confirm the fact that the Kramer technique adequately filled the dental pulp vessels. Teeth of various age groups etc., were studied.

Initially a standard ionic bone decalcifier was employed but was found to be too slow (four weeks plus). Accordingly a modified Schmorl technique was adopted utilizing a formic acid solution and heat; satisfactory decalcification for section purposes was then secured in 3 to 6 days. Minor modifications have been also made in setting up the Kramer apparatus (e.g., sealing medium, post injection tooth grinding).

Several unlabelled micro-photographs are enclosed to illustrate the ink-injected dental pulp vessels, and standard of the technique developed. The enlargement factor is given on the back of each photograph. These particular photographs emphasize the venous constituents of the dental vascular pattern although the actual slides can also be made to demonstrate the arterial features. Detailed studies of these vessels are proceeding.

Microradiographic studies to date demonstrate the entry of radiopaque material (e.g., thorotrast) into the major vessels, but the detail leaves room for further investigation. The microradiographic methods and technique are themselves being altered and different grain films used in an attempt to secure more definition.

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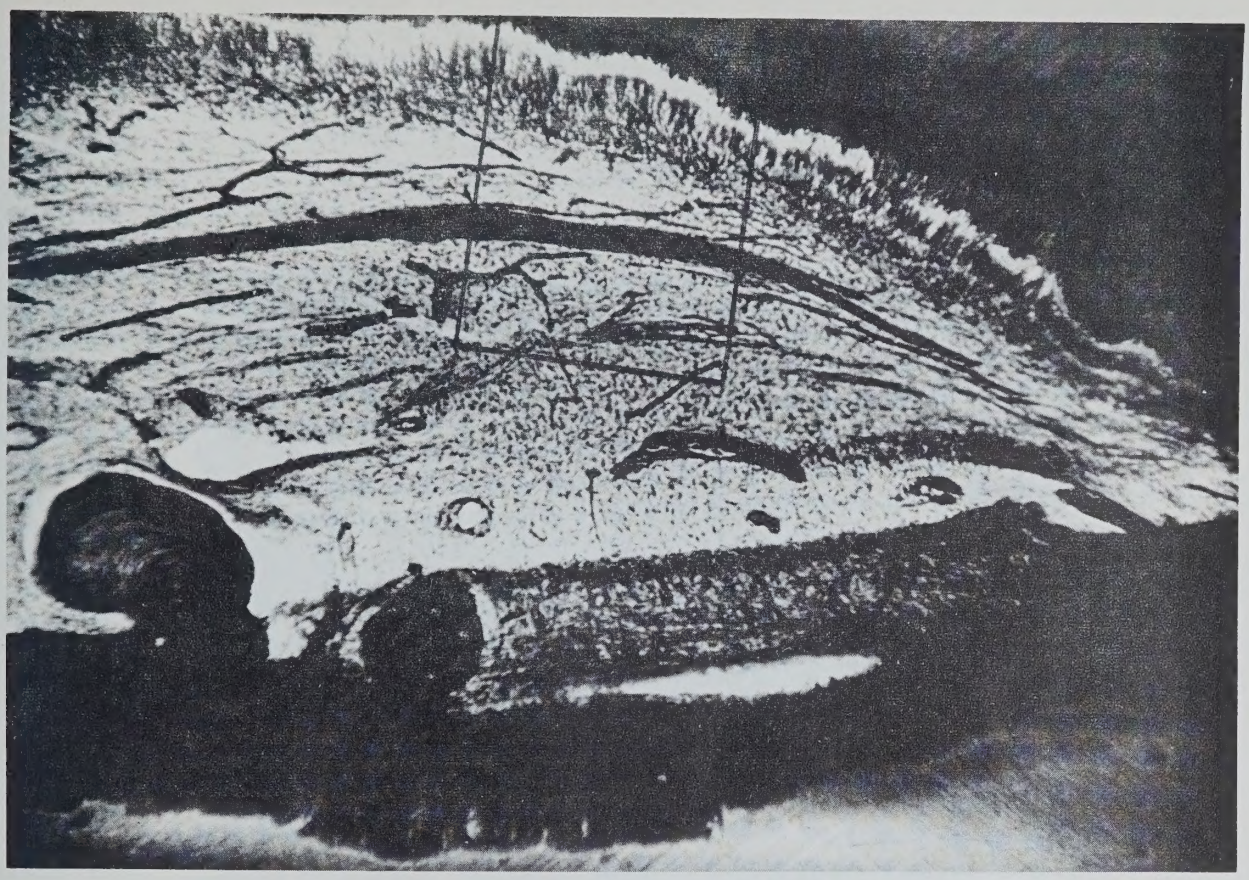
APPENDIX "A"



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APPENDIX "A"

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APPENDIX "B"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: The pathological characteristics of experimental fusospirochaetal infections.

Grantee: H.A. Hunter, Faculty of Dentistry, University of Toronto

Project No.: DR 47 Amount of Grant: $2200

SUMMARY OF WORK

12 cats, 12 rabbits, and 22 guinea pigs have been inoculated subgingivally, subcutaneously, and intravenously with pooled human gingival scrapings, guinea pig fusospirochaetal exudate and the filtrates of both. The gross and microscopic examination of these has led to the following results:

1. Lesions were produced only in cases where organisms were present.

2. The intragingival inoculation in cats produced only local acute abscesses that discharged spontaneously and left no permanent effect.

3. The subcutaneous guinea pig inoculations produced either local acute abscesses that healed, or a spreading cellulitis that ultimately killed the animal by means of a systemic toxaemia.

4. The intravenously injected rabbits developed lung and liver abscesses and the majority died of an intercurrent bronchopneumonia.

5. Attempts to demonstrate a toxic agent in filtrates of fusospirochaetal exudates or of pooled gingival scrapings were unsuccessful.

APPENDIX "B"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: The pathological characteristics of experimental fusospirochaetal infections.

Grantee: H.A. Hunter

Project: DR 47 Amount of Grant: $2200

The following report is a direct continuation of the report as given to this Committee in March 1953.

Histological Findings.

The tissues that were examined included the kidneys, lungs, liver adrenals, and skin. The last only where it was thought to be advisable. These were all routinely fixed in Zenker's formol solution, embedded in paraffin, and cut at 6 micra. Special Levaditi stained sections of liver were prepared from animals #53, 54, 55, 56, 61, and 62 which had received an intravenous injection of 0.1 ml. of guinea pig exudate.

In Group I (cats injected subgingivally) no significant tissue changes were observed that could be interpreted as deriving from the experimentally injected gingival scrapings or the filtrate of guinea pig exudate.

In Group II (guinea pigs injected subcutaneously) the following Table I had summarized the pertinent data.

In Group III (rabbits injected intravenously) the tissue changes observed are recorded in Table II.

The Levaditi stained liver sections did not show any spirochaetes, though the numbers examined were too few to be sure.

In the sections of abdominal skin overlying the spreading cellulitis, the most striking feature is the paucity of infiltrating leukocytes. They are present, but are largely confined to the immediate border of the layer of necrotic tissue that is both within and deep to the subcutaneous fat tissue. The cutaneous blood vessels are somewhat engorged, but are otherwise normal.

The preceding results indicate that lesions could be produced only by using inocula that contained viable microorganisms. The injection of filtrates of gingival scrapings and of guinea pig exudates either intragingivally, subcutaneously, or intravenously in no instance produced significant signs of disease.

The lesions that were produced when bacteria were injected took the form of either (a) local abscesses, (b) spreading

cellulitides developing at the site of injection; or, (c) pulmonary congestion in the intravenously injected rabbits. The broncho-pneumonia killed half the number of rabbits before the experimental period was over. This pneumonia was consequent to the toxaemia produced by the septicaemia. Several of these animals developed pyaemic abscesses in the liver and/or lungs. In the one form (a), they were sharply localized subcutaneous abscesses showing a good inflammatory reaction. In the other phlegmonous inflammation (b), the animals were quickly overwhelmed by a systemic toxaemia.

These results do not indicate the precise source of the toxin(s) responsible. They might either be bacterial in origin or derived from the injured host tissue cells. In this regard it must be borne in mind that to date no one has isolated an exotoxin from the fusospirochaetal group of organisms, nor have attempts to isolate and identify possible toxic tissue metabolites been successful. The failure of bacteria-free filtrates to produce lesions is suggestive of there being no exotoxins. Rosebury et al (1) tested unfiltered, centrifuged, supernatant from guinea pig exudate. They got negative results after subcutaneous injection of 0.2 ml. in four guinea pigs. This supernatant fluid showed widely scattered fusiform and other bacilli and cocci.

The explanation for the selective development of either a local subcutaneous abscess or a phlegmon involves the question of predisposition, but it appears to be partly quantitative.

In the case of the fusospirochaetal pyaemic abscesses in the lungs and liver of the rabbits, the source of the infection is known; i.e., intravenous inoculation, but they occur in natural life also. Smith (2) believed that most cases of lung abscesses are caused by aspiration, though three other routes are cited as possibilities. These are (i) infected emboli from some other focus, (ii) by way of the lymphatics and, (iii) direct penetration wounds through the chest wall.

Graham (3) reported that the injection of sterile filtrate of material scraped from the periodontal pockets of patients with "chronic pyorrhoea" caused injury to the liver and kidneys of animals. The use of chloroform as an anaesthetic followed by the autopsy report of central liver damage made it more difficult to attribute death to the experimental inoculum. In guinea pigs the presence of a slight amount of histologically demonstrable fat in the liver is acceptable as being within normal bounds, so that this lessens its importance as a factor in the death of the animal. We have been unable to duplicate Graham's results.

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1. Rosebury, T., Clark, A.R., Macdonald, J.B., & O'Connell, D.C. Jour. Infect. Dis. 87: 234-248, 1950.
2. Smith, D.T. Oral Spirochaetes and Related Organisms in Fusospirochaetal Disease. Williams & Wilkins, Baltimore, 1932, p. 153.
3. Graham, J.W. Proc. Roy. Soc. Med. (Odont.) 30: 51-58, Nov. 1936.

TABLE I

Animal	Inoculum	Site of Injection	Kidneys	Lungs	Liver	Adrenals
#65	1.0 ml. gingival scrapings.	Local abscess draining.	Normal	Normal	Slight visible fat	Normal
#66		Local abscess draining.	Hyperaemic	Normal	Slight visible fat	Normal
#67		Local abscess healing.	Hyperaemic	Normal	Slight visible fat	Normal
#68		Cellulitis	Granular degener.	Normal	Normal	Normal
#69	1.0 ml. filtrate of Scrapings.	Normal	Hyperaemic	Normal	Slight visible fat	Hyperaemic
#70		Normal	Normal	Normal	Slight visible fat	Normal
#71		Normal	Normal	Normal	Slight visible fat	Hyperaemic
#72		Normal	Normal	Normal	Slight visible fat	Hyperaemic
#73	0.1 ml. guinea pig exudate	Cellulitis	Granular degener.	Normal	Normal	Very hyperaemic
#74		Mult. abscess	Granular degener.	Early congest.	Granular degener.	Hyperaemic
#75		Cellulitis	Granular degener.	Early congest.	Granular degener.	Hyperaemic
#76 #76		Cellulitis	Granular degener.	Normal	Granular degener.	Very hyperaemic
#77	1.0 ml. filtrate of exudate	Normal	Normal	Normal	Granular degener.	Normal
#78		Normal	Normal	Normal	Slight visible fat	Slight hyperaemic
#79		Normal	Normal	Normal	Slight visible fat	Normal
#80		Normal	Normal	Normal	Granular degener.	Normal
#81		Cellulitis	No sections available.			
#82		Cellulitis	Granular degener.	Early congest.	No section	Hyperaemic

TABLE I (cont'd)

Animal	Inoculum	Site of injection	Kidneys	Lungs	Liver	Adrenals
#83	0.075 ml. guinea pig exudate	Cellulitis	No sections available.			
#84		Cellulitis	Granular degener.	Normal	Granular degener.	Hyperaemic
#85		Cellulitis	Normal	Early congest.	Normal	Normal
#86		Draining Local abscess	Normal	Normal	Normal	Normal

TABLE II

Animal	Inoculum	Site of injection	Kidneys	Lungs	Liver	Adrenals
#53	0.1 ml. guinea pig exudate	-	Hyperaemic Gran. degen.	Moderate congest.	Early fibrosis of portal areas. Central congent. Abscess	Normal
#54		-	Normal	Early congest.	Same as #53	Normal
#55		-	No section	Abscess. Widespread consolid.	Central congestion	Normal
#56		-	Granular degener.	Early congest.	Central fatty change	Normal
#61		-	Granular degener.	Moderate hyperaemic	Central congestion	Normal
#62		-	Normal	Abscess Consolid. Phlebitis	Same as #53	Normal
#57	1.0 ml. filtrate of exudate	-	Hyperaemic Slight	Slight hyperaemic	Normal	Normal
#58		-	Normal	Slight hyperaemic	Normal	Normal
#59		-	Slight hyperaemic	Slight hyperaemic	Normal	Normal
#60		-	Slight gran. degen.	Normal	Normal	Normal
#63	Controls	-	Normal	Normal	Normal	Normal
#64		-	Normal	Normal	Normal	Normal

"B"-5

APPENDIX "C"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: The effect of topical application of silicones in protecting teeth against acid solutions in vitro.

Grantee: Gordon Nikiforuk, Division of Dental Research, University of Toronto.

Project No.: DR 48 Amount of Grant: $2,240

SUMMARY OF WORK

Silicones are polymeric compounds composed of multiples of the structure $(R_2SiO)_x$, where R is the hydrocarbon radical. This chemical structure imparts to silicones a stable oxidized structure common to silica, and since certain R-Si linkages have been shown to be exceedingly inert and resistant to oxidation, it follows that the combination should provide thermal stability and resistance to many types of reagents to an extent not found in any of the organic polymers. The ability of these agents to form water repellent surfaces offers theoretical possibilities of preventing corrosion of teeth.

This report deals with the effect of topical applications of some silicone preparations in protecting teeth against acid solutions in vitro.

METHODS AND MATERIALS

The silicone polymers and related compounds tested included the following members: the methyl silicone oils; alkyl silicone resins; alkyl-aryl silicone resins; and methylchlorosilanes.

The original screening of some thirty silicone preparations was accomplished by applying the silicone to one-half of the tooth surface, using the other one-half as a control, and immersing the tooth in a 0.5 M. lactate buffer, pH 4.0, for one day. Silicones that protected surfaces against etching were further tested as follows: wax windows of standard dimension (approximately 3 mm. in diameter) were prepared on flat surfaces of non-carious teeth. The surface was then coated with a silicone preparation, dried for ten minutes in air, and then exposed to etching in 45 ml. of a 0.5 M. lactate buffer, pH 4, for six hours. Identical treatment on another surface of the same tooth formed the control. The degree of etching was assessed by staining with silver nitrate and by measuring the amount of calcium dissolved.

The effectiveness of several silicone preparations in preventing etching of teeth, in vitro, is summarized in Table 1. The most striking protection was offered by a group of alkyl-aryl silicone resins and by a methyl silicone resin.

The protection offered by silicone resins and oils against corrosion is due to formation of a thin film. With methylchlorosilane there appears to be a hydrolytic reaction with the methylchlorosilane vapour at the surface of the object being treated, depositing a thin film of methyl-polysiloxane which is a water repellent agent. Experiments are in progress to determine the life-span of the silicone film on a tooth surface when the tooth is continuously immersed in boiled saliva.

TABLE I

Wax windows of a standard dimension were prepared on flat surfaces of non-carious teeth. The surface was coated with a silicone preparation, dried for ten minutes and immersed in 45 ml. of 0.5 M lactate buffer, pH 4.0 for six hours. The etching was assessed by determining the amount of calcium dissolved and by staining with silver nitrate.

Types of silicone	mg of Ca^{++} dissolved	mg of Ca^{++} dissolved in control	% protection	Degree of etching - $AgNo_3$ stain
1. sodium methyl siliconate (2% solution)	0.734	0.772	4.9	++++
2. dimethyl silicone oils viscosity 0.65	0.536	0.628	14.6	+++
3. alkyl and alkyl-aryl resins;	.134	.336	61.0	++
a) methyl silicone				
b) alkyl-aryl resins:				
i) SR - 98	0.12	0.752	98.4	
ii) DC - 801	0.218	0.798	72.7	+
iii) DC - 803	0.010	1.000	99.0	
iv) DC - 804	0.022	0.924	97.6	
v) DC - 993	0.012	1.164	99.0	
vi) XR - 1296	0.618	0.718	13.9	++++
vii) DC - 2104	.008	.904	99.1	
4. chlorosilanes				
a) liquid	.466	.408	0.	++++
b) vapours dried for 3 1/2 days	.338	.408	17.1	+++
5. 2% NaF	.896	.912	1.8	++++
6. saturated Na_2SiF_6	.958	1.008	5.0	++++

TABLE I

Types of silicone	mg of Ca^{++} dissolved	mg of Ca^{++} dissolved in protein control	% Decrease of etching - $AgNO_3$ stain
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APPENDIX "D"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: The growth in width of the face and head of the Macaque monkey as revealed by vital staining.

Grantee: Dr. A.H. Craven, Dental School, McGill University

Project No.: DR 44 Amount of Grant: $1350

SUMMARY OF WORK

Two young macacus rhesus monkeys were injected with a 2% solution of alizarine red S. The time intervals were, one month between the first and second injection, and sacrifice one month later.

The two skulls were cleaned of soft tissue and embedded in clear plastic, (Castolite). Subsequently, one of the skulls was sectioned in a plane at right angles to the eye/ear plane by surface cutting with a shaping machine. The sites of staining were photographed as each new cut was exposed. The other skull was sectioned in the same plane by cutting with a band saw.

Growth sites contributing to increments of width in the face and cranium were discovered in these monkeys of a comparable age to a 6 year old child. In the cranium increments in width appeared due to increase at the sutures, while in the face both appositional and sutural growth appeared to play a part.

Further work is in progress to quantitate the increments of growth in the face and cranium of the rat. There has been a delay in that the machine originally proposed to section the skulls did not prove to have a large enough cut. Thus time has been spent in adapting a different sectioning method.

It is requested that further funds on this grant be held in abeyance, pending the possible appointment of the grantee to the University of New Zealand.

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October 1953

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Grantee: Dr. A.H. Craven, Faculty of Dentistry, McGill University

Project No.: DR 44 Amount of Grant: $1350

Introduction

In 1949, Moore published a very interesting paper on the determination of growth sites contributing to the antero-posterior growth of the cranium and face in the macacus rhesus. Unfortunately, the location of growth sites contributing to increase in width of the face and cranium was not examined. Thus, it was felt that the method outlined by Moore should be repeated and these contributors to width increments examined. The vital injections of alizarin red S stained the growing bone well and showed a few growth sites in the mid-line of the face and cranium.

Method and Material

Two macacus rhesus monkeys, with a dentitional age equivalent to that of a six year old child, were injected, intraperitoneally, with alizarin red S. The solution was administered as a 2% solution of alizarin in 0.45% NaCl. The dosage for the first injection was 10cc. for each animal, while the second injection, one month later, had a dosage of 15cc. for each animal. The animals neither gained nor lost weight during the period of this study. The larger animal weighed 2.78 kg., while the smaller weighed 2.28 kg. Both animals remained healthy until the time of sacrifice, one month after the second injection. The smaller animal (2.28 kg.) was maintained alive an additional week, following the ingestion of radioactive calcium-45.

Attempts were made to perfuse the animals with N.saline, under sodium pentothal, but difficulties were encountered in the friability of the arteries and veins. On examination, the viscera of the larger animal appeared healthy; but a small piece of kidney, preserved for sectioning, showed, histologically, a chronic inflammation. The right femur, tibia and fibula were dissected out and macerated by boiling in weak NaOH. The heads were removed and in the case of the larger animal macerated by quick immersion in conc. NaOH. The head of the smaller animal was stripped of soft tissue by dissection, there being no immersion in

/any aqueous solution. The alizarin stain was more evident on the cranium of this second animal.

Prior to embedding, the surface sites of deposition of radioactive Ca^{45} were examined by the method outlined by Jodrey and Wilbur (1951). Difficulty was experienced in the removal of the developed emulsion from the cranial vault and palate. The skulls were also examined and photographed before embedding in the plastic.

Castolite, as has been mentioned in a preliminary progress report, was used for embedding. Castolite has the consistency of glycerine and is mixed with a 'hardener' to give an appropriate setting time. It was found necessary to reduce the 'hardener' to the minimum, in which case layers of one inch thickness could be readily poured. Intermittent vacuum was maintained for 6 - 8 hours, but discontinued before gelation took place. The block of plastic was annealed at 70°F for 48 hours.

Sectioning of the larger animal followed the pattern outlined by Moore, namely, surface cutting. A slight modification was that instead of a screw cutting lathe a shaping machine was used. This machine gave a similar depth of cut. A 3/8-inch Carmet Style S-5 (Allegheny Ludlam Steel Corp.) carbide cutting tool was used. The plane of cut differed from that of Moore's study, in being a frontal cut at right angles to the eye-ear plane.

Each cut was photographed on 9 cm.X 12 cm. Kodak Commercial Ortho film at maximum magnification. The position of the camera was standardized. Significantly different cuts from the preceding ones were, in addition, recorded on Ektachrome colour film. A similar method to that used by Moore was employed to evaluate and illustrate the resultant photographs.

The head of the smaller animal was sectioned in the same plane as the other block, but by means of a band saw. Thus a series of permanent sections was obtained.

Findings

Gross examination of the crania: The specimens were examined grossly both before and after embedding. There was little, if any, loss of colour from embedding.

The Brain Case: The external surface of the vault exhibited stain along most of the suture lines. Figure 1. The width of this stain varied, being widest in the coronal and sagittal sutures and narrowest in the parieto-temporal suture. The suture lines, also, lay in variable relationship to the stained area, especially the sagittal suture of the first, or larger, specimen. The greater part of the staining of the lambdoid and coronoid sutures of the second, or smaller, specimen was on the parietal side. The amount of stain varied along these sutures, being greater toward the vertex.

The supraorbital ridges revealed faint staining, increasing in intensity toward the fronto-zygomatic suture. Figure 2. The temporal crest of the frontal bone showed faint stain.

A suggestion of surface staining was observed on the squama of the frontal bone in the mid line, about 2-3 cms. above the supraorbital ridge. Otherwise, the external surfaces of the bones of the cranial vault exhibited no stain.

As has been reported by Moore, the internal surfaces of the bones comprising the cranial vault exhibited a generalized staining; but large unstained areas were noticed, especially in the second specimen. These unstained areas seemed to correspond to the convolutions of the brain.

The base of the skull exhibited heavy staining on the body of the sphenoid bone and basilar portion of the occipital bone. Figure 3. The spheno-occipital synchondrosis was still present. The infratemporal surfaces of the great wing of the sphenoid and the temporal squama were heavily stained. The inferior surface of the zygomatic process and the articular eminence of the temporal bone exhibited stain. Surprisingly, the post glenoid process of both specimens revealed intense stain. The entire petrous portion of the temporal bone showed no stain. Even the tip was not stained. The jugular process of the occipital bone and the occipito-temporal suture showed stain.

The Face: In contrast to the brain case the face showed generalized surface staining. Figure 1. This surface staining was greatest in the premaxillary region and least on the anterior surface of the zygomatic bone. Surface staining showed a much less sharp distinction in the premaxillary-maxillary suture than in the maxillo-zygomatic suture, where the maxillary side seemed heavily stained. The fronto-zygomatic suture was stained and in this region surface staining appeared on both frontal and zygomatic bones toward the lateral side. Figure 2.

The orbits showed areas of heavy stain, the greatest being the orbital surface of the maxilla. Superiorly, the orbital surfaces of the frontal bone revealed localized patches of stain, similar to those reported by Brash (1934).

Posterior to the dental region, heavy staining was observed in the tuberosity. Figure 1. The alveolar border exhibited slight stain. In contrast to Moore's findings, the palates of both specimens revealed some mid line staining, especially in the posterior region. There was an intense surface staining of the alveolar process and palate lingual to the first permanent molar. Figure 3.

The Craniofacial Hafting Zone: The great wing of the sphenoid bone showed stain around most of the suture lines and on the infratemporal surface. The zygomatic arch showed surface stain on the lateral side. The zygomatico-temporal suture was also stained.

In contrast to Moore's findings, the pyramidal process of the palatine bone showed little surface staining. The medial pterygoid plate appeared relatively unstained, while the lateral pterygoid plate was well stained, especially along the posterior border. Figure 3.

The Mandible: This bone was not described by Moore, but was included here for the completeness of the study. There was a distinct difference in staining shown by these two specimens, notably along the inferior border of the body of the mandible. Figures 4a and 4b. The medial surfaces were similar and more heavily stained than the lateral, both in the body and ramus. The classic observations of resorbtion of the anterior border of the ascending ramus and deposition along the posterior border were supported in this study. A noticeable point, however, was that the deposition on the posterior border was mainly toward the lateral side. Figure 5. The gonial angle in both specimens showed no sign of stain.

Staining Revealed by Sectioning.
The Brain Case:

The first approach to the brain case was the sectioning of the supraorbital ridge of the frontal bone. The frontal bone in this region appeared very porous and the surface staining did not appear to extend through the inner table. The inner table of the frontal bone was heavily stained, but this inner staining was not even in distribution, patches of unstained bone were visible.

Subsequent sections revealed the superior surface of the orbital portion of the frontal bone to be stained, and in places extend through to the orbital surface. The junction of the great wing of the sphenoid, temporal and frontal bones revealed a site of heavy staining. Further posteriorly, this intense staining disappeared and was replaced by the relatively unstained parieto-temporal suture. The coronoid suture was well stained in its entire depth and the serial sections followed its ascent to the sagittal suture at bregma.

The sagittal suture showed intense staining along its entire length and appeared similar in each cut. The staining extended from the outer to the inner table.

Sections of the temporal bone showed no staining on the petrous portion, but stain on the internal surface of the squama. However, sections further back showed the mastoid portion between the occipital and parietal sutures, at this level both facing lateralward. Even further posteriorly the two parietal and the occipital bones showed heavy stain along the suture lines.

The body of the sphenoid, small wings of the sphenoid and basilar portion of the occipital bone showed stain throughout.

The Face:

The premaxilla was heavily stained, especially on the outer surfaces. Later cuts showed the premaxilla enclosed by extensions forward of the

maxilla. The premaxillary maxillary suture was stained but not heavily. The maxilla showed stain round the crypts of the unerupted teeth and in the sockets of the erupted teeth. The lateral surfaces of the maxilla as well as the alveolar margin showed stain. In contrast, the area of the maxilla bounding the nasal cavity appeared less well stained, but further toward the fronto-maxillary suture the intensity of the stain increased.

The more anterior cuts of the palate revealed little stain in the mid line and neither stain on the nasal nor oral surfaces. Further posteriorly, the stain in the mid line of the palate increased in intensity and the nasal surface of the palate showed stain. As had been observed externally, the palate in the region of the erupted first permanent molar was heavily stained.

The lateral staining of the zygomatic process of the frontal bone was evident in the sections. The fronto-zygomatic suture showed staining on both sides of the suture. Whereas the orbital surface of the zygomatic process of the frontal bone was unstained, the orbital surface of the zygomatic bone was stained.

The maxillo-zygomatic suture not only contributes to antero-posterior growth, as shown by Moore, but also to the lateral increments. Staining was observed mainly on the maxillary side of the suture. As has been observed previously the tuberosity of the maxilla was heavily stained, but it was also noted that the inclination of the tuberosity was downward and outward.

The Craniofacial Hafting Zone:

As had been noted superficially, the medial pterygoid plate was unstained, while the lateral pterygoid plate showed stain laterally. The hamular process of the medial pterygoid plate showed a small amount of stain.

The great wing of the sphenoid bone showed stain on both infra temporal and intracranial surfaces. The spheno-temporal suture was stained, as was the well stained spheno-frontal suture which at this level faced downward and outward.

On sectioning the outer surface of the zygomatic arch was heavily stained, but the inner surface showed no stain. The zygomatico-temporal suture was also stained. Sectioning also revealed the intense stain in the post glenoid process of the temporal bone.

The Mandible:

The greater part of the body of the mandible of the larger specimen was unstained, except for the sockets of the teeth and the alveolar margin. Sections of the ascending ramus revealed more stain on the lingual surface. The tip of the coronoid process was stained. Sections of the condylar head showed intense stain beneath the articulating surface, the heaviest stain being medialward.

The findings in this study support the observations of Moore (1949) and Massler and Schour (1941), in that the sutures are sites of bone growth in the cranial vault and that the external surfaces reveal little stain. No explanation could be found for the isolated patches of stain observed on the frontal bone. On the other hand, the sites of muscle attachments showed stain, e.g., the temporal crest. The unstained areas of the inner surface of the skull did not appear to be a remoulding of the shape of the bone, but rather projected a little beyond the rest of the surface. It appeared as though the inner surface of the vault was being smoothed out.

The distribution of stain observed on the supraorbital ridge and zygomatic process of the frontal bone supported the observations of Keith and Campion (1922) that surface deposition was the chief contributor to width in this region. But the staining of the zygomatic bone was in contrast with deposition medially. It seemed that the orbit retained its shape without the distortion which would occur by movement of the zygomatic bone laterally by the deposition of the stain on the orbital surface of the zygomatic bone.

As has been observed by many investigators the lateral movement of the zygomatic arch was due to deposition laterally and resorbtion medially.

The observation, by Moore, that the petrous temporal bone was being withdrawn from the space between the basilar portion of the occipital bone and the great wing of the sphenoid, seemed to be supported by this study. On the other hand, no visible stain was present at the tip of the petrous portion. The impression gained from this study was that the whole temporal moved laterally. Indeed, this would not be illogical if the glenoid fossa were to be adjusted to lateral mandibular growth.

The heavily stained post glenoid process seems to be part of the development of the temporomandibular joint which continues growing for a long period after birth. Sicher (1949) illustrates the continued growth of the post glenoid process in the human.

The greater deposition of stain on the medial end of the mandibular condyle and on the lateral side of the posterior surface of the ascending ramus appear to be adjustments to width increments which would occur by growth of the posteriorly diverging rami.

The relatively unstained medial pterygoid plate and well stained lateral plate seem to offer evidence that the width of the nasopharynx is established early and remains relatively unchanged. In contrast the tuberosity of the maxilla increases in width by the downward and outward divergence.

The palate showed a greater amount of stain, in the mid line, posteriorly, and would seem to suggest that the change in arch form of the macacus rhesus, reported by Baume and Becks (1950), could, to a large extent, be due to this growth. On the other

hand, change in intercanine width of the maxilla appears to be a combination of sutural (premaxillary maxillary suture) and surface growth. In Moore's study, the use of a slightly acid methyl methacrylate removed some of the surface stain. In general, it appears that surface addition plays a relatively large part at this stage of development of the face and dentition.

Conclusions

The use of alizarin red S as a vital stain has revealed in the Macacus rhesus of dental age equivalent to the human at six years of age:-

1. Further evidence that the cranial vault increases largely by sutural addition, both vertically and laterally.

2. The increase in width of the dental and facial structures is due to both sutural and surface addition.

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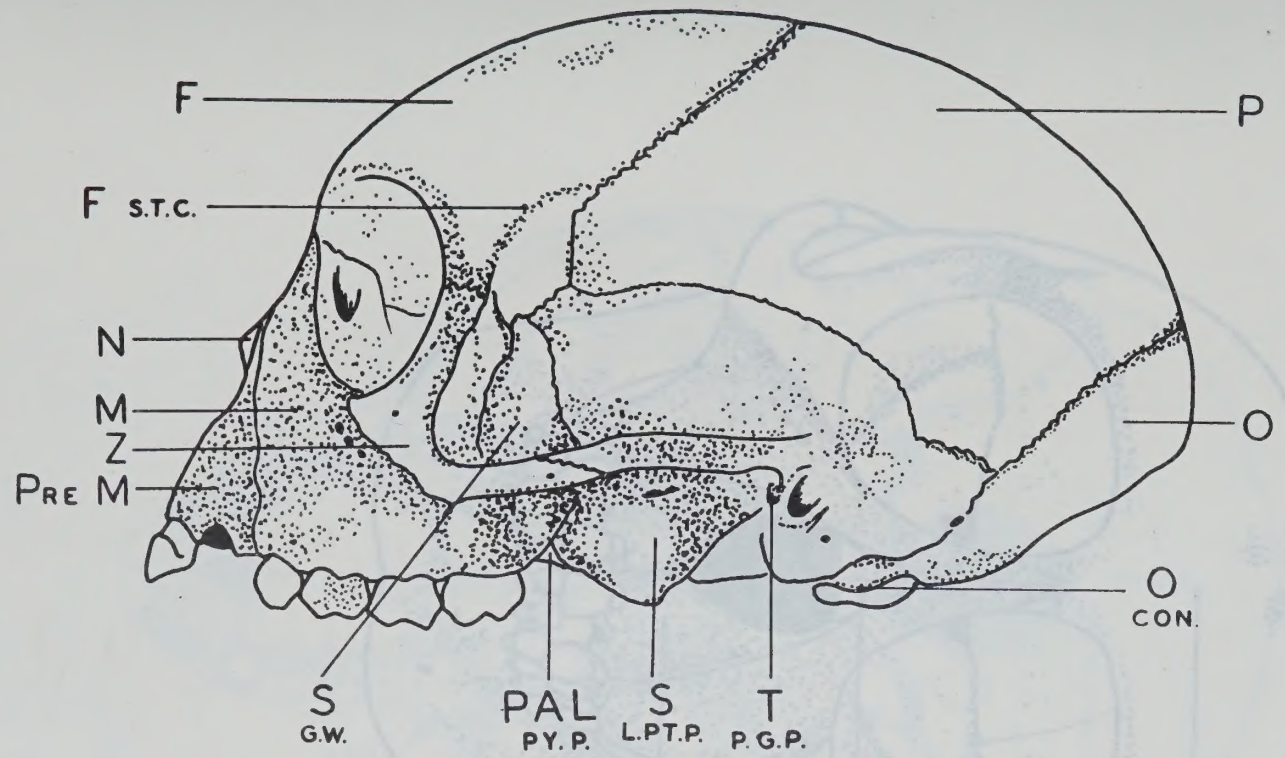
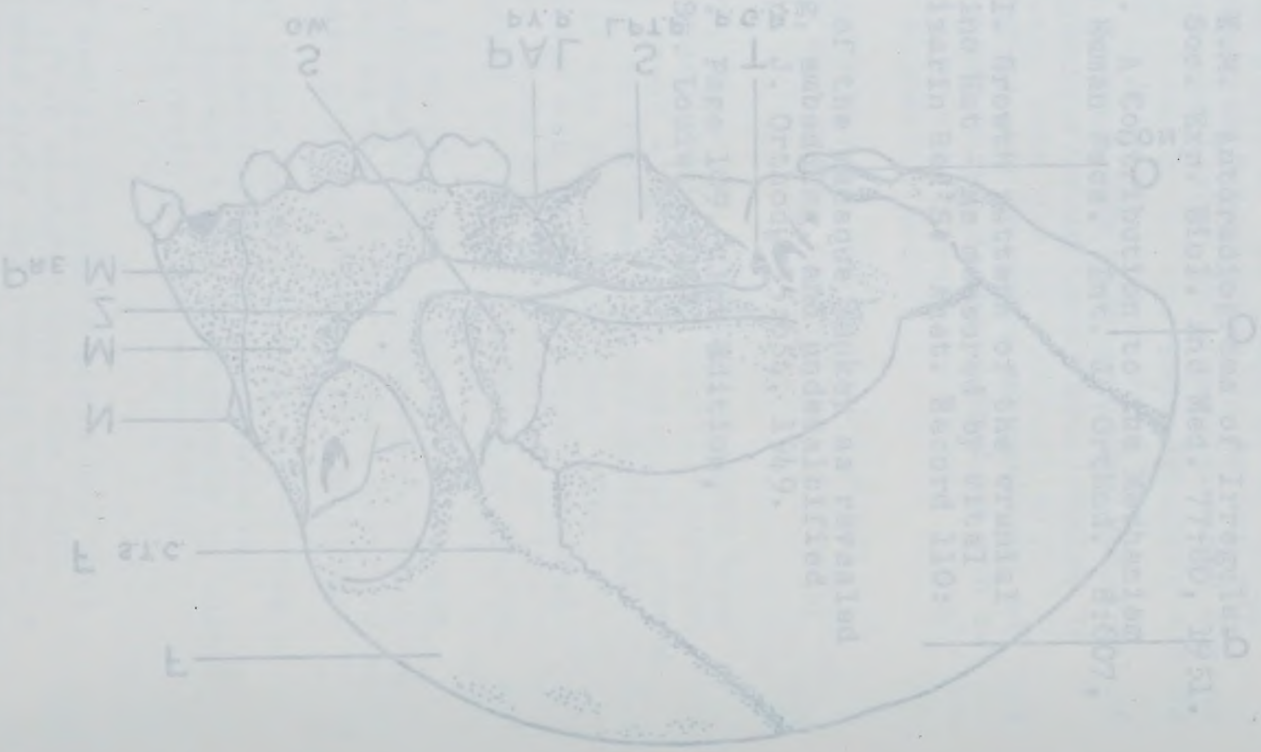


Figure 1. Lateral view of the skull of the larger specimen. The sites and intensity of the stain are shown by the stippled areas. F, frontal bone; F s.tc.,temporal line on the frontal bone; M, maxilla; N, nasal bone; O, occipital bone; O con., occipital condyle; O j.p.,jugular process of the occipital bone; PAL, palatine bone; PAL py.p., pyramidal process of the palatine bone; P, parietal bone; Pre M., premaxilla; Sb, body of the sphenoid bone; S.g.w., great wing of the sphenoid bone; S.l.pt.p., lateral pterygoid plate of the sphenoid bone; S.m.pt.p., medial pterygoid plate of the sphenoid bone; T.pet.,petrous portion of the temporal bone; T.p.g.p., post-glenoid process of the temporal bone; Z,zygoma.



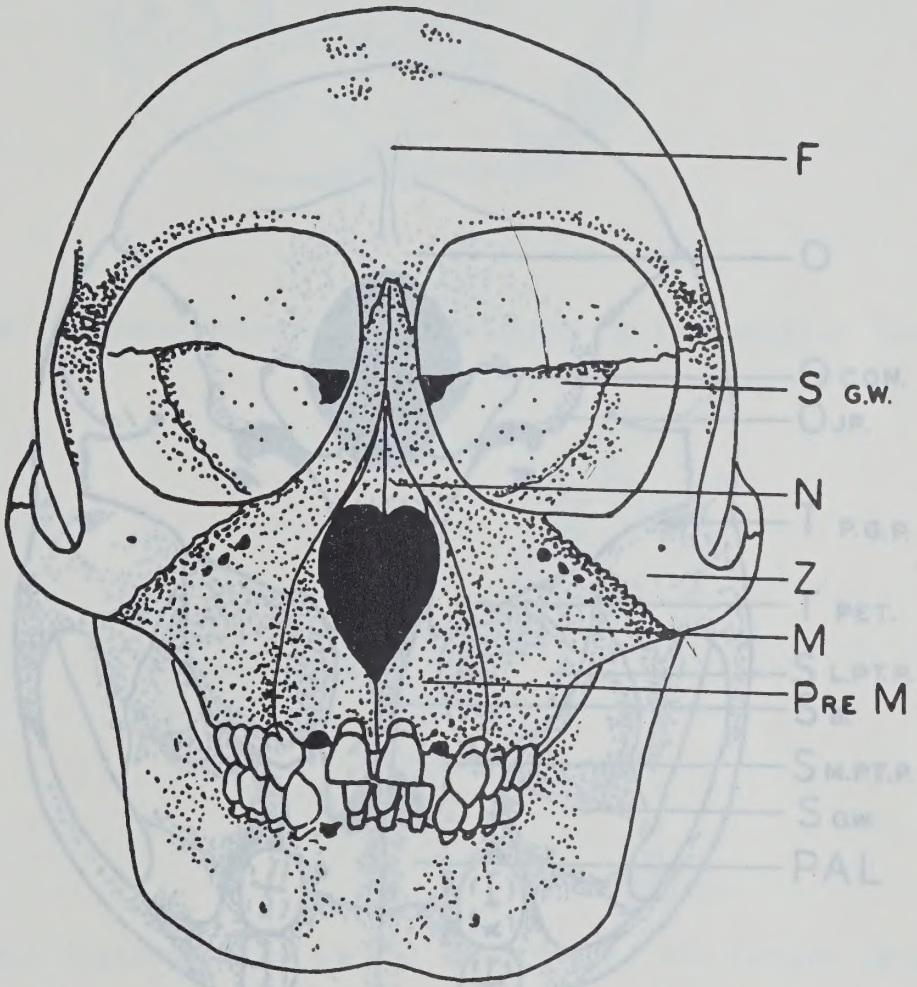


Figure 2. Frontal view of the same skull.

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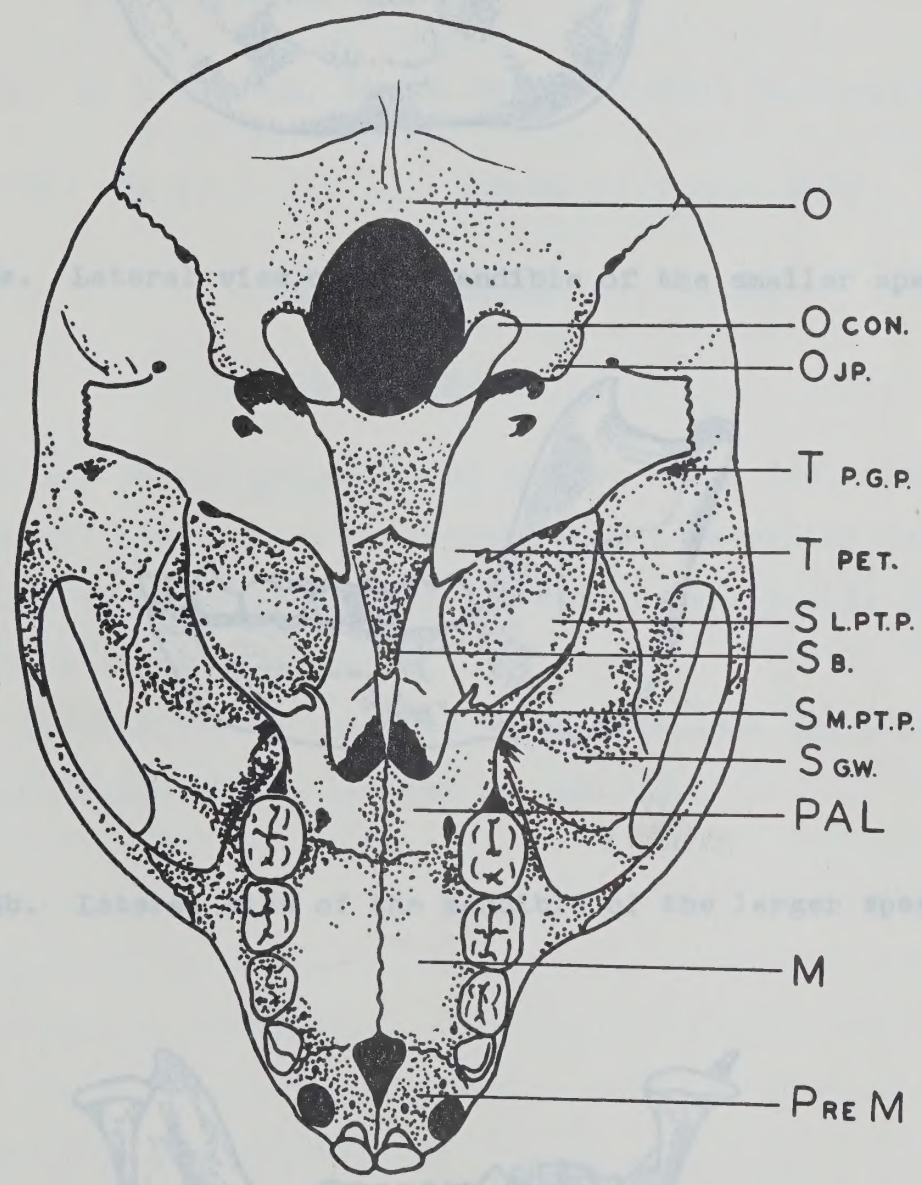

Figure 3. Inferior view of the skull.

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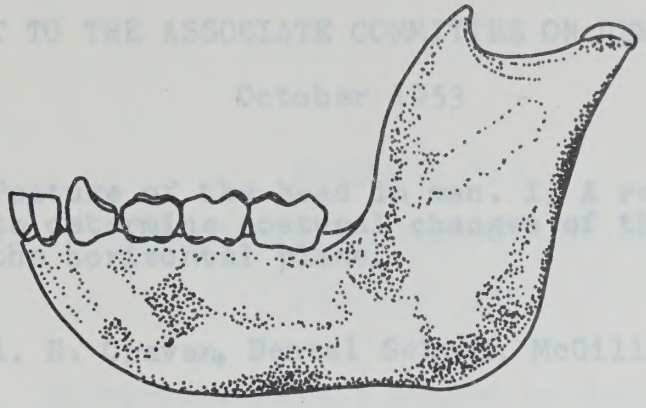


Figure 4a. Lateral view of the mandible of the smaller specimen.

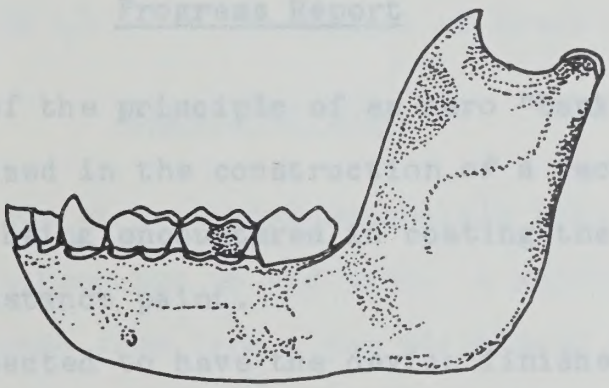


Figure 4b. Lateral view of the mandible of the larger specimen.

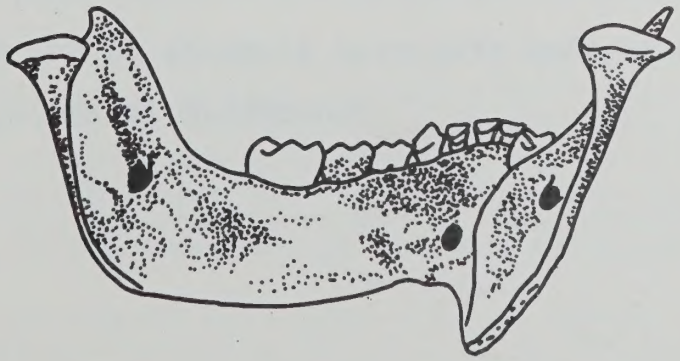
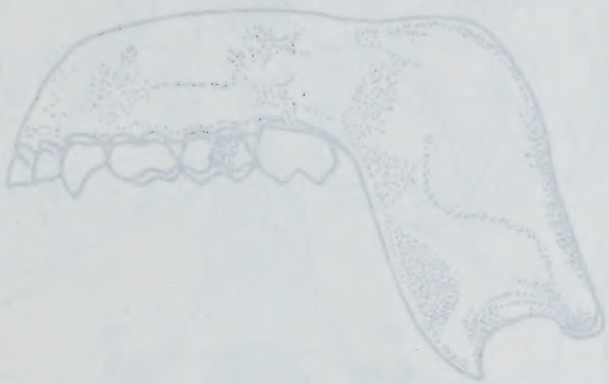
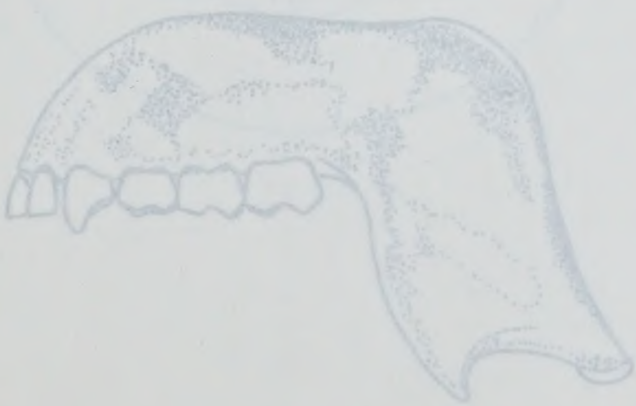


Figure 5. Medial view of the mandible of the larger specimen.





APPENDIX "E"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1953

Subject: Posture of the head in man. I. A recording device to determine postural changes of the head in the horizontal plane.

Grantee: A. H. Craven, Dental School, McGill University

Project No. DR 49 Amount of Grant: $400

SUMMARY OF WORK

Progress Report

The use of the principle of an aero "bank indicator" has been utilized in the construction of a recording device. Difficulty is being encountered in coating the inside of the tube with resistance paint.

It is expected to have the device finished and a final report available before the end of the year.

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APPENDIX "F"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: Electropotentials caused by dental alloys

Grantees: S.G. Davis, Operative Dentistry,
University of Alberta

H.R. MacLean, Department of Chemistry,
University of Alberta

Project No.: DR 43 Amount of Grant: $1025

SUMMARY OF WORK

Two methods of measuring potentials with a minimum of current flow have been set up and used. A galvanometer which measures the current flow from a one microfarad condenser which has been previously charged by the source of potential was used as one method. A Keithley vacuum tube electrometer was also used.

Potential differences were measured between alloys found into plastic buttons and suspended in Ringer's solution at 37° C. So far no attempts have been made to reduce the size of the potential difference.

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Project No.: DR 43 Amount of Grant: $1025

The aim of this project is to measure the potential which exists between different dental alloys such as are used in the mouth. The methods of using the alloys will be varied to reduce this potential and thus prevent any effect of large potential differences in the mouth. A knowledge of the potentials produced by combinations of alloys will enable one to choose sets of alloys which will give only a small potential.

Two potential-measuring instruments which require little or no current to operate them were used. A Leeds and Northrup #2239 galvanometer was used in a condenser-ballistic galvanometer potential difference meter. This meter was assembled and calibrated using a type K potentiometer, a standard cell and a potential divider. In this instrument a one microfarad condenser is charged by the unknown potential and then this condenser discharged through the galvanometer. The deflection of the galvanometer is proportional to the potential difference. This instrument may be read to the nearest 0.5 millivolt.

The second instrument used was a Keithley vacuum tube electrometer which requires only 10^{-14} emperes to operate. This instrument can only be read to the nearest 10 millivolts.

These two instruments were used to minimize polarization effects due to the flow of current necessary to operate the voltmeter.

Specimens to be tested were made up in small plastic buttons which had a small hole drilled through them. This hole was filled with amalgam so that it could contact electrolyte at the bottom, and a platinum contact could be made at the top surface of the amalgam. The measurements were made with these buttons dipping into a U tube containing Ringer's solution. The assembly of buttons and U tube were maintained at 37°C in a constant temperature bath.

Various procedures have been used in making the measurements. The platinum leads connecting the alloys to the potential measuring instrument were contacted to the dry surface, and to the surface with a drop of Ringer's solution.

Commercial amalgams have been tested so far and potentials up to 245 millivolts have been noted. Some difficulty has been experienced in obtaining the composition of these amalgams.

Typical results are listed in the following table.

Potentials between Commercial Dental Amalgams

In Ringer's solution at 37°C - dry contact

	Potential in millivolts
20th Century and Lang's White Beauty	10
" " " Cresilva	15
" " " Fellowship	15
" " " True Dentalloy	45
Cresilva and Lang's White Beauty	20
" " Fellowship	10
" " True Dentalloy	45
Fellowship and Lang's White Beauty	10
" " True Dentalloy	55
True Dentalloy and Lang's White Beauty	50

These potential differences are small due to the similarity in composition of the amalgams. A potential difference was noted between a "wet" pack and a "dry" pack. A wet pack of True Dentalloy of 7½ of Hg to 5 of alloy measured against a dry pack of 5 to Hg to 5 of alloy gave a potential of 40 millivolts.

The results indicate that potentials do exist between even similar dental alloys. It is proposed to extend the measurements to include other alloys. Difficulty is still experienced in making the measurements due to the polarization of the surfaces involved and due to static charges.

APPENDIX "G"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: The isolation and characteristics of Bacteroides nigrescens

Grantee: J.B. Macdonald, Faculty of Dentistry, University of Toronto

Project No.: DR 50 Amount of Grant: $400

SUMMARY OF WORK

Twelve strains of gram negative anaerobic rods which produce black colonies on blood agar have been isolated. The technique found suitable for isolation involved cross-streaking with a hemolytic strain of Micrococcus aureus. With one exception, satellite colonies developed in a zone about 1 cm. wide around the staphylococcus streak.

The strains do not grow in conventional fluid media. However, when 20 per cent of a filtrate of a 24 hour broth culture of Micrococcus aureus was added to the fluid media good growth was obtained. The growth factor from this staphylococcus culture was found to be heat stable. It could not be replaced by yeast extract.

It was thought possible that the growth promoting effects on blood agar plates might be associated with the release of hemoglobin from erythrocytes as a result of hemolysis. To further examine this point the strains were cultured on chocolate agar, and on agar prepared with blood which had been hemolyzed by alternate freezing and thawing. In neither case was growth found to be satisfactory.

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Potentials Between Commercial Dental Amalgams

Subject: The Isolation and characteristics of Bacteroides nitrescens

Grantee: J.B. Macdonald, Faculty of Dentistry, University of Toronto

Project No.: DR 50 Amount of Grant: $400

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APPENDIX "H"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: Mechanism of bone and tooth formation in the light of autoradiography and histochemistry.

Grantee: Leonard F. Bélanger, School of Medicine, University of Ottawa.

Project No.: DR 52 Amount of Grant: $1200 (Supplementary to the award made by Division of Medical Research)

SUMMARY OF WORK

In continuation of our studies of the mechanism of mineral deposition, the organic portion of mineralized tissues will be the main concern of the investigations during the current year. Three groups of young rats (6-8 in each group) and one group of young hamsters (6 animals) were injected subcutaneously with S^{35} separated isotope in form of weak solution of sulfuric acid. The procedure for fixation and autoradiography has been that described in the attached paper. Preliminary results were presented at the Nineteenth International Physiological Congress in Montreal and summarized as follows:

"S^{35} as H_2SO_4 separated isotope was introduced subcutaneously into 4 day-old rats which were then sacrificed at intervals of 1, 2 and 24 hours and on alternate days up to 8 days thereafter. Autoradiographs were prepared by the inversion technique from alcohol-formaldehyde fixed tissues and from sections of same, pretreated with formic-citrate at pH 4.9 or incubated with hyalyronidase. The cartilage showed an intense accumulation of S^{35} in the proliferating cells at 1-2 hours. The sulphur was then seen in the capsule and cartilage matrix at 24 hours and by the end of the second day it was mostly in the areas of hypertrophy and calcification indicating growth movement towards the epiphyseal plate. An overall weak persistent reaction was recorded also over cartilage, tendons, ligaments and aponeuroses. The initial picture over bone was one of general, rather large intake. After 2 days only a small autoradiographic band at the periosteal junction and a weak reaction over the new spicules were apparent. In teeth, there was also an overall intense reaction over dentine, which had disappeared after 2 days, to make place for a weak band which migrated with

time towards the dentino-enamel junction. Over enamel there was a strong initial linear reaction at the ameloblast border, which moved slowly and diffusedly towards the inner regions of enamel with time.

After acid demineralization, the autoradiographic picture persisted over cartilage and areas of growth in bone, dentine and enamel. The overall initial reaction over bone and dentine disappeared with the minerals.

With hyalyronidase incubation, the reaction disappeared rapidly from bone and dentine and slowly from cartilage according to a pattern probably indicative of the original concentration. The picture persisted over enamel after it had disappeared from dentine, bone and cartilage."

"H-3"

(*Reprinted from Nature, Vol.* 170, *p.* 625, *October* 11, 1952)

Improvements to the Melted Emulsion Technique of Autoradiography

THE method whereby autoradiograms are obtained by covering histological sections with photographic emulsion[1] has been improved or modified for specific needs[2–7]. In our laboratory, the inversion technique[3] has gained considerably in definition and ease of operations by substituting the emulsion from Kodak High Contrast Positive film for that of Matrix film (Eastman Kodak Co.) which contains a yellow dye.

The emulsion from Kodak High Contrast Positive film is unstained. It is removed easily from the base under the relatively bright Series 0*A* 'Yellow-Green Safelight'. It is very fluid at 38° C. and does not retain air bubbles. It can be processed and washed at the usual temperature (20° C.). It has a better resolution (165 lines per mm.)[4]; shows fewer background artefacts, and can be used for contact, for coating stained specimens[1,2,5], or for unstained sections which are inverted and stained after the photographic processing[3].

The technique at present in use for tracer studies of the mineralization of bones and teeth is as follows. For contact autoradiograms, strips of 35-mm. film are cut to appropriate size and placed in contact with the histological preparation, previously coated with 1 per cent celloidin in alcohol-ether. These are placed between two glass slides bound with cellulose tape and exposed in the dark at 4° C. After processing, the film strips are again bound between two glass slides with cellulose tape. This makes handling, storing, inspection and identification easy. In the melted emulsion technique, strips of 35-mm. film are cut to appropriate size (6–8 in.) and hydrated for a few seconds in distilled water, at room temperature. Each strip is then laid down on a glass plate, emulsion face up, and the emulsion is scraped from the central area with a glass slide. The emulsion is collected in a small beaker at 38° C. and, when melted, is picked up with a clean medicine dropper and transferred to the celloidin-coated microscope preparation. The practice of coating with a 'Blanchard brush'[3] has been altogether abandoned because it produces uneven coats and favours the production of air bubbles. The emulsion is then gelled and dried on a levelled glass plate, under a gentle air stream. The dry specimen is exposed in the dark at 4° C.

Development can be carried out at 20° C., but the

cold bath (10° C.) for 10 min. gives less background fog and fewer artefacts. The formulæ *D*11 or *D*19, diluted 1 to 1 with distilled water at the time of use, give a more accurate picture than the previously used *D*72 ('Dektol').

The preparations are then rinsed in distilled water at 10° C., fixed for 10 min. in Kodak acid fixer with hardener also at 10° C. and finally washed under a gentle stream of tap water for 30 min. They can be dehydrated, cleared and mounted at this stage or inverted under water[3], remounted on a glass slide, section face up, and dried under a gentle air stream. For staining these inverted preparations, a 0·02 per cent aqueous solution of basic fuchsin is used, after passing through fresh oil of origanum and down-graded alcohol solutions to distilled water. Basic fuchsin solutions keep well and seem to gain strength when stored in glass-stoppered brown bottles; an older solution will stain well in 5 min., while a fresh one will require up to 10–15 min. Sections are transferred rapidly through 95 per cent alcohol, which differentiates and removes excess stain from the background. They are then passed through three baths of absolute alcohol. For clearing, oil of origanum is preferred to xylene or other light oils; it is messy but minimizes artefacts. Two baths are used and the preparations are finally mounted in 'Permount' (Fisher), using liberal amounts.

This work was carried out under a grant from the Advisory Committee on Medical Research of the National Research Council of Canada. The radio-elements were provided by the Council's establishment at Chalk River, Ontario.

Leonard F. Bélanger

Department of Histology and Embryology,
School of Medicine,
University of Ottawa.
July 15.

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APPENDIX "I"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: Chemical mechanisms in dental caries

Grantee: J.J. Rae, Department of Chemistry, University of Toronto

Project No.: DR 1 Amount of Grant: $1680

SUMMARY OF WORK

The research on ammonia production was concluded this summer by Miss H.M. Shemilt. The inhibitory action of glucose on ammonia production by saliva was found to be caused by a lowering of the pH. The stimulatory action of glucose on urea-saliva mixtures can also be attributed to the fact that the glucose keeps the pH near the optimum for ammonia production for a longer time. A draft of a paper to be submitted to the Journal of Dental Research will follow.

Lactic acid production and the inhibitory substance in bromoform-treated ground tooth enamel are still being investigated but there is no new evidence on these topics to be presented at this time.

APPENDIX "I"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: Chemical mechanisms in dental caries

Grantee: J.C. Ray, Department of Chemistry,
University of Toronto

Project No.: DR I Amount of Grant: $1650

SUMMARY OF WORK

The research on ammonia production was concluded this summer by Miss H.M. Shmidt. The inhibitory action of glucose on ammonia production by saliva was found to be caused by a lowering of the pH. The stimulatory action of glucose on urea-saliva mixtures can also be attributed to the fact that the glucose keeps the pH near the optimum for ammonia production for a longer time. A draft of a paper to be submitted to the Journal of Dental Research will follow.

Lactic acid production and the inhibitory substance in bromolorm-treated ground tooth enamel are still being investigated but there is no new evidence on these topics to be presented at this time.

APPENDIX "J"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: Experimental fusospirochetal infection

Grantee: Dr. J.B. Macdonald, Division of Dental Research,
Faculty of Dentistry,
University of Toronto

Project No.: DR 35 Amount of Grant: $3650

SUMMARY OF WORK

The phase of this study dealing with reproduction of experimental infections of guinea pigs has been successfully completed. The following is a summary of a paper now under preparation.

1. The exudate from a serially passed guinea pig fusospirochetal infection has been bacteriologically analyzed.

2. The resultant 16 pure bacterial cultures and a pure culture of spirochetes were recombined and cultivated together by a new technique.

3. The recombined cultures in three trials were successfully used to produce transmissible fusospirochetal infections in guinea pigs.

APPENDIX "J"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: Experimental fusospirochetal infection

Grantee: Dr. J.B. Macdonald, Division of Dental Research,
Faculty of Dentistry,
University of Toronto

Project No.: DR 35 Amount of Grant: $3650

SUMMARY OF WORK

The phase of this study dealing with reproduction of experimental infections of guinea pigs has been successfully completed. The following is a summary of a paper now under preparation.

1. The exudate from a aerially passed guinea pig fusospirochetal infection has been bacteriologically analysed.

2. The resultant 10 pure bacterial cultures and a pure culture of spirochetes were recombined and cultivated together by a new technique.

3. The recombined cultures in three trials were successfully used to produce transmissible fusospirochetal infections in guinea pigs.

APPENDIX "J"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: Experimental fusospirochetal infection

Grantee: Dr. J.B. Macdonald, Faculty of Dentistry, University of Toronto

Project No.: DR 35 Amount of Grant : $3650

REPORT

This report constitutes a section of a paper prepared for publication. II. Recombination of strains

Procedure:

Nine attempts were made to recombine together all 16 bacterial strains from HG plus a "pure" culture of spirochetes from HG. The experimental design was essentially the same for all nine trials with modifications in individual trials as noted below. It was planned that animal inoculations would be made only in those cases where the results indicated that all strains had grown in the recombination.

All cultures (except spirochetes) were grown initially from ampules stored in CO_2 ice. They were inoculated into Difco thioglycollate broth, and as streaks on Proske-Sayers (PS) blood agar, and incubated anaerobically for seven days, i.e., the incubation period required for the slowest growers. The growth was then recorded for both tubes and plates, and all cultures were examined microscopically. Where growth in broth was satisfactory, broth cultures were used for recombination. Otherwise growth from blood agar was used. If growth failed in both media an ampule was removed from CO_2 ice storage and used for the recombination.

Spirochete cultures were obtained in two ways. They were grown in PSAFKE agar in Noguchi tubes using as source either pure spirochetes previously isolated and stored in CO_2 ice or the spirochete haze surrounding confluent bacterial growth on PS blood plates inoculated with HG guinea-pig exudate. Alternatively, the spirochete haze from PS blood plates inoculated as unstreaked drops with HG guinea pig exudate was emulsified with PSAFKE broth and used directly for the recombination studies. In the latter case, some of the emulsion was inoculated into spirochete tubes of PSAFKE agar to confirm viability and inoculated as streaks onto blood agar plates for both aerobic and anaerobic incubation to confirm purity. Spirochetes from the first source (i.e., pure spirochete growth in Noguchi tubes) were used in the first, fourth, fifth, sixth, and eighth trials. Emulsified spirochetes from blood plates were used for the second, third, seventh, and ninth trials.

Cultures were recombined on PS blood agar plates. A drop of PSAFKE broth was placed in the centre of each plate. Using a wire loop each pure culture was inoculated in turn onto the plate as a single streak from the edge of the plate into the drop of broth in the centre of the plate. The order of inoculation of the various strains was the same on all plates. When all 16 cultures had been inoculated each streak appeared as the spoke of a wheel with the central drop in which all cultures were merged forming the hub. Spirochetes were inoculated directly into the central drop without being streaked.

Each seed culture was in addition sub-cultured to Difco thioglycollate broth, and to two blood agar plates for both aerobic and anaerobic cultivation to confirm purity. Thawed guinea pig exudate from the eleventh guinea pig passage was inoculated as unstreaked drops on PS blood agar and was streaked on blood agar for aerobic incubation.

Except for the aerobic blood plates all cultures including the recombination plate were inoculated anaerobically in modified Brewer's jars for seven days. The aerobic plates were incubated three days.

The recombination plates were examined after seven days and the growth of each strain along its individual "spoke" was recorded. In most instances heavy growth was found along the length of the streak and into the central drop. In some instances growth would stop short of the central drop. Sometimes a strain failed to show any growth. Where all strains grew along the whole length of the streak, the agar containing the mixed growth in the central drop was cut out asceptically and emulsified in a tissue grinder with PSAFKE broth, approximately 1 ml. of broth for each drop. After dark field examination for emulsion was inoculated as unstreaked drops on fresh PS blood agar (for anaerobic incubation) and streaked on blood agar for aerobic incubation. The mixed growth of guinea pig exudate was similarly emulsified and subcultured.

After seven days incubation the recombination drops of mixed growth on blood agar were examined grossly, emulsified in a tissue grinder as before and examined by dark field. In those trials where growth appeared comparable grossly and by dark field examination to the mixed growth of guinea pig exudate on blood agar, and where there was no evidence of contamination, the emulsified growth was inoculated into guinea pigs. The mixed growth of guinea pig exudate was inoculated into guinea pigs as a control.

Except for the first trial three plates were used in recombining the cultures at each trial. In the first trial thirty plates were used. The intention here was to emulsify the central drops and inoculate directly into guinea pigs without further culture.

In the third and fourth trials the blood agar used for streaking into the recombination drops was poured into plates made with a bottom of Visking dialysis tubing instead of glass.

These plates had been previously set into ordinary blood agar plates inoculated with guinea pig exudate according to a technique described elsewhere. The purpose of this modification was to permit dialysis of nutrients from growth of unpurified exudate to the area of recombination.

RESULTS:

In the nine trials growth without contamination of all 16 bacterial strains plus growth of spirochetes was obtained only twice - i.e., Trial 8 and Trial 9 (Table 2). In Trial 3 all strains grew (including spirochetes) except strain K100. In four other trials (first, second, sixth, and seventh) certain of the strains failed to grow, and in three of the trials (fifth, sixth, and seventh) contaminants were found on one or more of the plates. Trial 4 was unsatisfactory because of running together of the cultures caused by water of condensation. The latter resulted from the necessity of incubating the dialysis plates without inverting. Growth did not appear to be enhanced in any respect in trials three and four as a result of using plates made with dialysis tubing.

The central growth, where the sixteen streaks merged was emulsified and subcultured in the first, third, eight, and ninth trials. In the first trial a culture of spirochetes from a Noguchi tube was added to the emulsion before it was inoculated as unstreaked drops onto fresh blood agar. After incubation growth appeared comparable to mixed growth of guinea pig exudate from the control series except that there was no spirochete haze and no spirochetes were found by dark field examination.

In the third, eighth, and ninth trials the growth of emulsified recombination drops exhibited a well-defined spirochete haze ranging in diameter from 5 to 15 mm. The bacterial growth was dark brown in colour and pebbled. A narrow zone (0.5 mm.) of creamy radially streaked growth surrounded the central portion. Beyond this was a flat granular pink-green irridescent margin from 0.5 to 2 mm. wide. The spirochete haze was beyond this. The zone of pink and green irridescent growth, by dark field examination appeared to be composed solely of strain K80 (motile rods). The control cultures of guinea pig exudate appeared similar to those of the recombined cultures except that they had no irridescent margin.

III Pathogenicity of recombined cultures.

Procedure:

The mixed growth from six recombination drops from the third, eight, and ninth trials was emulsified with PSAFKE broth (1 ml. to each drop). Four normal guinea pigs were inoculated with each emulsion in a dosage of 1 ml. Inoculations were made subcutaneously into the groin without trauma. The emulsion was also streaked aerobically on blood agar. In addition a control culture of unpurified guinea pig exudate grown on blood agar was emulsified and similarly inoculated into four guinea pigs. The animals were examined daily and their condition recorded. Exudate was aspirated whenever possible, examined by dark field, and streaked on blood agar for aerobic incubation. Exudate from each of two

animals of the experimental group was passed to two more animals in each series using a dosage of 0.075 ml. Exudate from only one guinea pig in the control series was passed to two more guinea pigs using a dosage of 0.075 ml. Exudate from two of the second passage animals was passed serially through three more passages in all three experimental groups using a dosage of 0.05 ml. Four animals were used at each passage in each experimental group. Exudate from only one animal in the control series was passed except as noted in Table 3. The exudate on each occasion was examined by dark field and streaked on blood agar for aerobic incubation. All guinea pigs were autopsied when found dead or sacrificed and autopsied not later than eight days following inoculation.

Results:

The results are recorded in Table 3. Lesions developed in every guinea pig in all five passages and in all three experimental groups. The lesions were in every respect comparable to earlier descriptions of experimental fusospirochetal infections in guinea pigs. Localized lesions varied in size from about 0.5 cm. in diameter to about 4 or 5 cm. in diameter. The smallest consisted of small fibrotic nodules with little or no exudate. There were only three of this type - one in the fourth passage of Trial 3, and two in the fifth passage of Trial 8. Larger localized lesions were well walled-off abscesses containing abundant thin to thick foul-smelling exudate. The colour varied from a creamy yellow to a dirty grey-brown. The walls of these abscesses showed extensive necrosis. Dark field examination of the exudate revealed a typical fusospirochetal flora including actively motile spirochetes of the T. microdentium variety, motile rods like strains JV5 and K80, non motile rods, fusiform rods and cocci. Lesions of this type were found in 14 of the guinea pigs from Trial 3, 14 from Trial 8, and 10 from Trial 9.

Lesions in which the original abscess was not successfully walled-off spread up the abdominal wall between the skin and the peritoneum causing extensive necrosis of tissue. In those cases where the animals survived for seven days there was generally some attempt to localize the lesion. The margins showed fibrotic thickening in some instances and in some there was a deep red inflammatory border -- about 1 to 2 cm. wide. In the two instances in which extensions proved fatal in seven days or less there was no evidence of any attempt to localize the infection. At death the lesions were found to cover most of the abdominal and thoracic walls; there was extensive necrosis of tissue and the exudate was thin and grey-brown in colour. The animals lost weight, and appeared moribund for about one day before death. Necrosis of skin with alopecia occurred over the abdomen of these animals and some animals in the group which showed non-fatal extensions.

Lesions in the control group of guinea pigs which were inoculated initially with a mixed culture of unpurified HG exudate appeared comparable in kind to those in the experimental groups. However, they were more severe. Nine of the guinea pigs developed spreading infections which were fatal in seven days or less and only four developed localized non-fatal abscesses.

Data bearing on this difference in severity appears in the following section.

The apparent decrease in severity of the experimental infection in Trials 8 and 9 from the fourth passage as shown in Table 3 was not borne out in subsequent passages. Exudate was passed serially from the fifth passage (Trial 8) through seven more passages in a dosage of 0.1 ml. as shown in Table 4. No change in severity of the infection was noted.

DISCUSSION

Methods used in screening the pure cultures isolated from the HG exudate were similar to those used by Rosebury, Clark, Macdonald and O'Connell in analyzing a different exudate (MP). It seems worthwhile to compare the results of the two analyses.

The total number of distinctive strains isolated from each exudate was approximately the same (15 from the MP exudate and 16 from HG, exclusive of spirochetes), and the morphologic categories were comparable. However, organisms were isolated from each exudate which clearly had no counterpart in the other exudate. Examples include the strain of Bacteroides nigrescens, the motile gram negative rod, K80, from the HG exudate and a strain presumed to be Spirillum sputigenum (VB7A) from the MP exudate.

These differences make it necessary to conclude that all the organisms which persist in serially transmitted fusospirochetal infections in guinea pigs are not essential to the infection or else that these comparable infections from different sources are caused by different groups of organisms. Obviously these conclusions are not mutually exclusive. Further assessment of the role of individual organisms must await further study.

The difficulty experienced in attempts to recombine the cultures isolated from HG lend support to the hypothesis that earlier attempts to infect guinea pigs with recombined cultures failed because some of the recombined cultures failed to grow or were nonviable. Growth of all cultures appeared to occur in only two of the nine recombination trials. In one other, (Trial 3) only one strain failed to grow (strain K100). This particular strain failed to grow in several of the trials (See Table II). Spirochetes failed in only two of the nine trials.

Contamination was also a problem because of the large number of times each plate had to be opened for streaking. Contamination was minimized by the use of a hood during the streaking operation.

The possibility of loss of virulence of individual strains as a result of prolonged cultivation was ignored. Each strain was subcultured frequently enough to provide as good adaptation to the artificial media as seemed likely to occur before the recombinations were attempted.

The technique of recombination provided good evidence regarding the viability of each strain in the recombination mixture and it is practical enough to permit easy repetition.

The experimental infections fulfilled all the conventional requirements for fusospirochetal infection including necrosis of tissue, production of foul-smelling exudate, typical fusospirochetal flora by dark field examination and transmissibility from animal to animal.

Strain K100 appears to have been not essential to the infection since pathogenicity in Trial 3 where it was missing was comparable to that in Trials 8 and 9. The successful production of these infections with recombined pure cultures opens the way to further studies aimed at identifying the significant organisms in these complex mixtures.

The demonstration of increased virulence with serial passage through guinea pigs of unpurified fusospirochetal exudate is significant in respect to the occurrence of acute fusospirochetal infections such as Vincent's angina. As pointed out by Rosebury evidence has been lacking to support the widely held clinical impression that Vincent's stomatitis is communicable. It has also been pointed out that experimental fusospirochetal infections have been comparable in severity whether the human source was a fatal noma or a mild gingivitis. This has been interpreted as supporting the view that virulence of the bacteria involved remains the same regardless of source. The present findings suggest a need for further examination of this question to determine whether virulence of these mixed infections can be enhanced regularly by serial passage through animals.

TABLE 2

Results of Nine Trials at Recombining Strains from HG Guinea Pig Exudate

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9
Plate 1	N.G. spirochetes ✱	N.G. spirochetes	W.C.	W.C.	W.C.	cont.	N.G.-K100 cont.	Satisf.	Satisf.
Plate 2	N.G. spirochetes	N.G. spirochetes	N.G. K100	W.C.	W.C.	cont.	N.G.-K98 K100 K110 K92 K80	Satisf.	Satisf.
Plate 3	N.G. spirochetes	N.G. spirochetes	N.G. K100	W.C.	W.C. cont.	N.G. K100 JB_3A K80	cont.	Satisf.	Satisf.

Key: N.G. = no growth W.C. = water of condensation on plate

cont. = plate contaminated satisf. = plate satisfactory, all stains grew

✱ K92 and/or K100 failed to grow in 25 of 30 plates in Trial 1.

"J"-7

APPENDIX "J-8"

Table 3. Pathogenicity of Recombined Cultures in Guinea Pigs

Passage	Inoculation	Trial 3 Recomb.	Trial 8 Recomb.	Trial 9 Recomb.	Control
1	1.0 ml.(cult. mash)	+	+	+	+
		+	+	++	+
		+	++	++	++++
		+	++	+++	++++
2	0.75 ml.(exudate)	++	+	+	+
		+++	+	+	++++
		+++	+	+	
		++++	+	+++	
3	.05 ml.(exudate)	+	++	+++	++++*
		+	+++	+++	++++*
		+	+++	+++	++++
		++	++++	+++	N.S.**
4	.05 ml.(exudate)	±	+	+++	+
			+	+++	++++
		+	++	+++	
		+++	+++	+++	
5	.05 ml.(exudate)	+	±	+	++++
		+	±	+	++++
		+++	+	+	
		+++	++	+	

Key: ± = small nodular lesion. No exudate
+ = less than 1" localized abscess
++ = localized abscess 1" or more in diameter
+++ = localized abscess with extension. Not fatal in 7 days
++++ = spreading infection fatal in 7 days or less
* = animals died before exudate aspirated
** = non specific death in one day.
No evidence of infection.

Table 4 Pathogenicity of exudate from recombined cultures (Trial 8) through guinea pig passages 6 to 12

Passage	6	7	8	9	10	11	12
Lesion	+ +++ + +++	+ +++	+++ +++	± +++	+++ +++	+ +++	+ +++

See Key in Table 3

Table 5 Survival of guinea pigs during 7-day period following inoculation with HG exudate

Passage	Dosage 0.050 or 0.075 ml.		0.10 or 0.15 ml.	
	No.of guinea pigs dead	No. of guinea pigs alive	No.of guinea pigs dead	No.of guinea pigs alive
1 to 7	1	3	19	36
8 to 15	23	14	15	2

Table 4 Pathogenicity of exudate from recombined culture (Trial 8) through guinea pig passages 6 to 12

Passage		6	7	8	9	10	11	12
Lesion		+	+	+++	±	+++	+	+
		+++	+++	+++	+++	+++	+++	+++
		+++						

See Key in Table 3

Table 5 Survival of guinea pigs during 7-day period following inoculation with HG exudate

Passage	0.050 or 0.075 ml.		0.10 or 0.15 ml.	
	No. of guinea pigs dead	No. of guinea pigs alive	No. of guinea pigs dead	No. of guinea pigs alive
1	23	14	15	2
2			19	6

APPENDIX "K"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: Entry of phosphate, carbonate and calcium into teeth, as shown with the help of radioactive elements.

Grantee: Dr. C.P. Leblond, Department of Anatomy, McGill University

Project No. DR 23 Amount of Grant: $3000

SUMMARY OF WORK

The general orientation of the present work has been to compare the deposition of the mineral elements and the formation of the matrix in the tooth of the rat. It was intended to carry out this work using both dentine and enamel. So far, only the data obtained with dentine lend themselves to precise conclusions.

Deposition of the mineral elements of teeth

The work on this subject was carried out with the help of radio-phosphorus and radio-calcium (See enclosed report by Miss Yurika Kumamoto). The main conclusions are that the deposition of mineral elements takes place in the dentine in an area immediately in contact with predentine. This does not seem to be a mere apposition on the inner surface of dentine, but the material appears to be deposited in a narrow, although definite region beyond the predentino-dentinal junction.

In addition, a gradient of deposition spreads out from that area outwards, that is, into the substance of dentine. This 'interstitial' formation of dentinal minerals exists to some extent in animals of all ages, but the younger the animals, the more pronounced this phenomenon is.

The third feature of mineral deposition is that the band of reaction occurring in animals sacrificed soon after injection persists as new layers of dentine are added. However, the reaction band may become somewhat diffuse and irregular -- a fact suggesting that some re-arrangement of the crystals of dentine may occur after their formation.

In previous reports we had emphasized only two features of mineral deposition in dentin, namely, formation of new crystals occurring in an appositional manner and exchange between the atoms at the surface of the crystal and those present in solution. The present results indicate that, in addition to these two phenomena, there may be formation of new crystal material within the dentine (interstitial deposition) and furthermore that formed crystals may eventually be re-arranged. Work on the cause of this re-arrangement is being continued.

Formation of the organic matrix of bone.

This is being investigated by means of radioautography after injection of radio-carbon. This work is detailed in an article with R.C. Greulich, copy of which is enclosed. It appears that the matrix of the dentine is one of the most stable components of the body.

Conclusions with regard to the formation of dentin.

The labelled component of the dentinal matrix after injection of C_{14}-bicarbonate has to be a substance of large molecular weight, such as the collagen which is known to exist in this organ. In addition to collagen, the matrix appears to contain mucopolysaccharides which may be detected histologically by metachromatic staining with toluidine blue or by the reactivity with the periodic acid-Schiff technique. The predentine which stained little or not with these two techniques may consist of collagenous material only. Therefore, the mucopolysaccharides of dentine must appear in dentine at the predentino-dentinal junction, that is, where also the mineral elements are deposited. It is tentatively suggested that the dentinal mucopolysaccharides may be necessary for the deposition of the dentine crystals and may, in fact, bind these crystals to the collagen framework.

APPENDIX "K"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1953

by

Yurika Kumamoto

DEPARTMENT OF ANATOMY, McGILL UNIVERSITY, MONTREAL

Re: Project DR 23

DEPOSITION OF MINERAL SALTS IN GROWING TEETH AS SEEN BY RADIO-AUTOGRAPHY

Since the February 1953 report, in which the results obtained from one series of Ca^{45} injected to 3-day old group was given, emphasis was laid on the 3-day old rats. In the present report, 3-day old rats were utilized to confirm an experiment with Ca^{45} carried out earlier. In addition, P^{32} injection was given to two rats. To determine the fate of double injections, two rats were injected on the 3rd and 6th day; the result will be elaborated later.

Materials and Methods

A group of black and white hooded rats were injected at 3 days of age with a solution of $Ca^{45}Cl_2$ and sacrificed at 1 hour, 1, 3, 6, 7, 8, and 9 days after injection.

Two rats of 3 days of age were injected with a solution of $H_3P^{32}O_4$, and sacrificed 3 hours and 1 day after injection.

Two rats were injected with Ca^{45} on the 3rd and 6th day of life and sacrificed on the 9th and 11th day of life, i.e., they were sacrificed on the 3rd and 5th days after the second injection.

All injections were by the subcutaneous route. The dose for Ca^{45} was 1 μc per gram of body weight or about 5 μc in 0.05 cc per rat in the case of these which received only a single injection, and correspondingly larger for the second dose. For phosphorus, the dose was 30 μc per rat in 0.154 cc.

All rats were sacrificed under chloroform anaesthesia. Their jaws and teeth were removed and immediately fixed in an alcoholic 10% formaldehyde fixative (3 parts 95% alcohol: 1 part 40% formalin) at a pH of 7 - 8.

Unstained and alizarin stained section of the molars and incisors were made by the paraffin and celloidin methods and later coated with emulsion in the radio-autographic technique.

Another method was tried to obtain sections of hard teeth. In this method, the tissues after fixation were dehydrated and immersed in ethylene dichloride or ether-alcohol (1:1). The tissues were then glued onto microscope slides with generous amounts of polymerized methyl methacrylate dissolved in ethylene dichloride or by clear Revlon nail polish in the latter case. After thoroughly drying the adhesive, the jaws were ground on a revolving carborundum wheel, which was attached onto an Atlas Drilling Press. In this arrangement, the carborundum wheel was horizontal, so that a pan of water was placed below, in which the wheel revolved. In this way the radio-active dust from the specimen was kept from contaminating the air and also it kept the surface being ground from being over-heated. When one surface was ground to a desired place which was determined by examination under the dissecting microscope, it was washed in tap water, dried and removed from the slide. In most cases, the adhesion onto the slide was not too solid and the tissues were easily removed. When the adhesion was solid, a scalpel was used while a drop of water was allowed to creep underneath the tissue, in much the same way as a colloidon bag is separated from the wall of a test tube. The cleaned dried tissue was then glued onto the slide, this time with the ground surface down over the glass. After the glue had dried, the grinding was carried out again, and when the section appeared transparent under the 100x magnification of the microscope, the sections were considered thin enough and the edges of the glue, which had also served as the embedding material for the tissue, was sealed with more nail polish. These unstained preparations were then subjected to the autographic method of analysis.

Observations and Results

In both the molar and incisor teeth, the autographic reaction over the dentine was concentrated in the newly formed dentine adjacent to the predentine at early time intervals (Fig. 1). This reaction was shown in the autographs as a band of reaction which at later time intervals was inside the dentine due to the fact that the new dentine added after the time of injection did not contain as much radio-active minerals. This band of reaction which is at first sharply delineated becomes somewhat diffuse with time. (Figs. 1, 2, 3 and 4).

In the experiments with the newborn group described in an earlier report, the band was found to disappear or become lost at later time intervals after injection. However, in the present observations in the 3-day old rats, it was found that the line of reaction representing the deposition of the radio-element became diffuse with time but persisted in about the same location at 9 days after injection in the molars (Fig. 4) and 12 days after injection in the case of the upper incisors (Fig. 5, and corresponding photo).

The dentine of the rats injected twice with radiocalcium recorded the two injections which showed in the autographs as two bands of reaction. In the apex of the molar cusp, where the distance between the bands is widest, it was found to be 55.17 μ. This when converted to the daily growth is equivalent to 18.4 μ per day. (Figs. 6 and 7).

At all times, the enamel showed only one form of reaction, consisting of two gradients in the autographic reaction. Even in those rats which received two injections the enamel did not register the two injections separately. The reactions over the enamel were always lighter than over the dentine. There was an increase in the number of reduced silver grains which constitute the autographic reaction from the base of the tooth to the incisal or occlusal portions, and this was called the basal-incisal (for the incisor) or the basal-occlusal (for the molar) gradient. The second, the radial gradient, decreased from the dentino-enamel junction to the outer surface of the enamel.

In the 3-day old group, the reactions were similar in the dentine and enamel. This was in contrast to that observed in the newborn group in which the dentine of the incisor only showed the band reaction in the autographs.

Radiophosphorus and radiocalcium autographs were identical in the deposition of the isotopes over both the enamel and dentine. (Fig. 8).

Discussion

The fate of the minerals incorporated in the dentine at the time of injection in the 3-day old group was studied. All the minerals were found to be deposited in the layer of dentine adjacent to the predentine. General histochemical stains such as hematoxylin-eosin, and Mallory's triple stain for connective tissues, show the uptake of the dyes to increase sharply at the predentin-dentine border and continue so in the dentine. Metachromasia has also been demonstrated to increase sharply at this junction, and remain so in the dentine. Some change therefore, takes place at this junction, whereby the mineral salts become embedded and retained in the dentine matrix.

Since the animals selected for the present study are extremely young they are in rapid growth. During the experiment, the dentine increased in thickness pulpwards. The further accumulation of mineral salts over the surfaces containing the radio-active salts incorporated immediately after injection results in the condition in which the band of radio-active salts become further and further away from the pulp. The salts deposited immediately after the injection are radio-active but those deposited later do not contain much radio-activity. This results in a diffuse reaction which is found on the pulp side of the band of reaction in the dentine. Thus, relatively speaking, the band "seems" to move through the dentine towards the dentino-enamel junction, although it is really stationary. This was found to be the case in the 3-day old group. Ocular micrometer measurements of the distance between the band of reaction and the dentino-enamel junction in these animals up to 3 days after injection in 3 locations, namely at the tip, the labial side, and the lingual side, revealed that the distances were not significantly changed. Similar measurements of ground preparation of upper molars showed the site of the band of reaction to be unchanged up to 12 days after injection.

In the case of the newborn rats, it was found that the band was lost after an interval. It is interpreted at present that the salts of dentine in the newborn group are not as stable as in those of the 3-day old.

With time after injection, the sharpness of the autographic band decreased. Several possible explanations are available for the observation.

One of the reasons is the interstitial deposition of more labelled minerals in the dentine. This would add to the overall diffusion of the original band reaction. A possible source for the labelled atoms would be those which escaped the bone during its remodelling. This is a logical source for the specific activity of the blood decreases markedly after injection. It has been found that the teeth become exceedingly more difficult to section during the course of the experiment, a definite proof that there is an accumulation of more salts.

Another explanation is the ionic exchange of the salts of the mineral of the dentine. This would result in the diffusion of the original band reaction. This does not however, take into account that the teeth increase in hardness.

Yet another reason, is that of a local solution and dissolution of the mineral salts due to some mechanical factors such as pressure and tension. It is hardly likely that the dissolved salts would be deposited in the same orientation as before, and this would result in the diffusion of the band reaction. Again, this phenomenon alone would not account for the increasing hardness of the tooth.

The deposition of the mineral salts which showed in the autographs as a band is a demonstration of the incremental nature of the deposition of the mineral salts. This was clearly shown in the autographs obtained from animals injected twice with radiocalcium. In these animals sacrificed 3 and 5 days after the second injection of Ca^{45} at 6 days of age, (first injection at 3 days of age), two bands of reactions were clearly visible. These were found to be 55.17 μ apart, and by simple calculation, it was found to be 18.4 μ per day. Such a figure, although arrived at from only two measurements, is interesting in itself since, the value compares well to those obtained by other methods such as after alizarin (Hoffman and Schour, 1940), strontium (Weinmann, 1942) and fluoride (Schour and Smith, 1934) injections.

Another fact of interest was that in these rats, which had received two injections of labelled atoms, the second molar showed a diffuse radioautographic reaction in the dentine as well as a band reaction. The first injection has been deposited in the pattern of two gradients, the basal-occlusal and radial gradients. The second injection was recorded in the form of a band, which indicates that there was enough matrix at the stage of incorporating the minerals that a band was formed.

The enamel at all times showed similar autographic reactions, which indicated that its mineralization proceeds slowly and is not subject to the changes in the environment as is the dentine. This was shown in the fact that although two injections were given to rats, the pattern did not differ from those which had received only one dose of radio-activity.

The reaction was always lighter than that over the dentine. This can be explained since the enamel formation occurs in two phases, the matrix formation and matrix maturation phases. It has been analyzed that in the former phase there is some 25% of the total calcium content of the adult enamel, whereas during the maturation phase, the rest of the mineral salts (75% of the total Ca content) become incorporated (Weinman et al., 1942; Wasserman, 1944; Marsland, 1952). Therefore, it is not surprising that the radio-elements incorporated into the enamel at the time of injection did not exceed that in the dentine; after all, the rats were only 3 days of age, and the second phase would barely have begun at the time of injection. In fact, Mallory's triple stain, which shows the enamel in 3 different stages of development, the last of which is the beginning of the maturation phase, in the 3-day old rat molar shows that the latter has just begun to commence.

It has been noted that the enamel is less fragmented in the histological preparations than dentine. If it is assumed that fragmentation occurs due to hardness and brittleness due to mineral salts, then, it is clear that at the ages selected for observation, the enamel was not mineralized.

Conclusions

1. The previous conclusion that predentine does not incorporate mineral salts has again been confirmed in experiments using 3-day old rats. This means that some change must take place at the predentin-dentine border to make possible the deposition of mineral salts in this area.

2. The similarity of Ca^{45} and P^{32} autographs under the present experimental conditions has again been confirmed.

3. The manner of deposition of mineral salts is different in enamel and dentine. The deposition of minerals in the enamel is a somewhat diffuse process, occurring to a slight extent at the matrix formation stage and to a considerable extent in the maturation stage.

Dentine is deposited in an appositional manner, the daily rate in the 3-day old rats being 18.4 μ (which compares favourably with those obtained by other methods).

4. The length of time after injection in which the presence of the band reaction in the 3-day old group is visible has been extended by the use of the grinding method. The results show that in the 3-day old at least there is no change in the distance of the band reaction from the dentino-enamel junction. This may not be so in the case of the newborn rats. It may be that the dentine salts deposited in the newborn may not be as stable as those deposited when the animals are 3 days old.

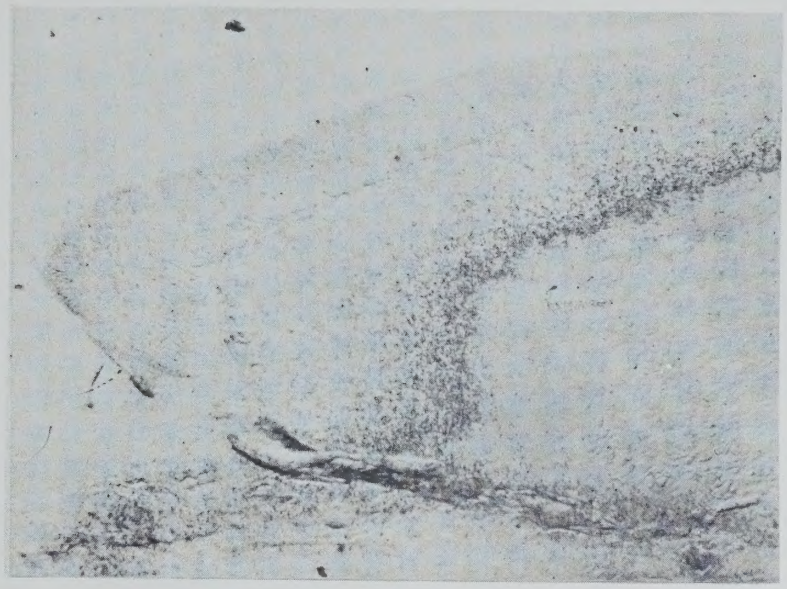
The loss in the sharpness of the autographic band reaction with time in both groups of animals has been ascribed to one or a combination of the following explanations: interstitial calcification after the initial deposition of the labelled atoms, or a rearrangement of the atoms of the mineral salts.

FOOTNOTE

1. The work done with Ca^{45}, P^{32} has been submitted in a M.Sc. thesis August, 1953.

FIGURES

Fig. 1.

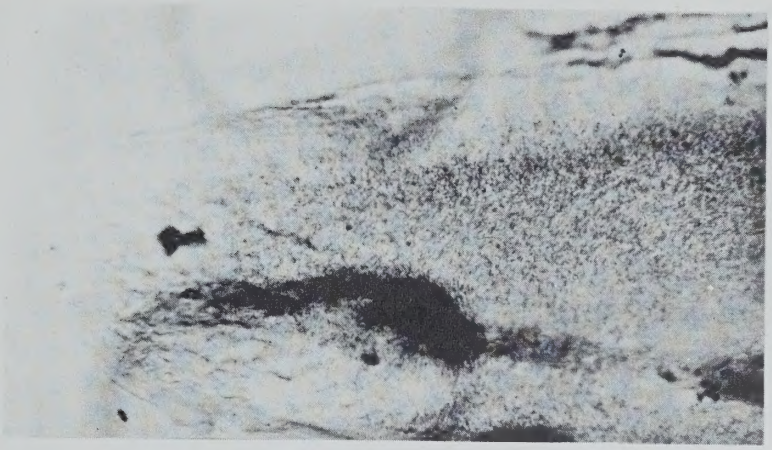


Coated radio-autograph of a lower incisor of a 3 day old rat 1 hour after injection of $Ca^{45}Cl_2$. The radiocalcium is deposited in the dentin adjacent to the predentin. Over the enamel there is no band reaction.

Fig. 2

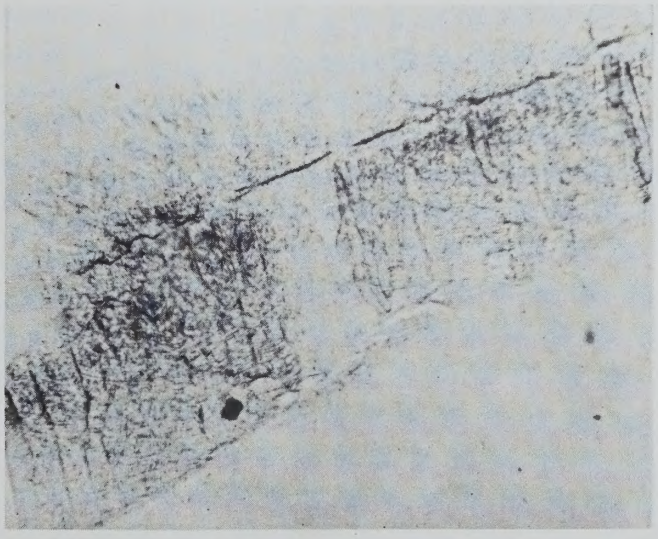
Coated radioautograph of a lower incisor 3 days after injection of Ca^{45} to 3 day rat. The reaction is now seen inside the dentin because during the subsequent 3 days after injection of the isotope, additional dentin was laid down. That laid immediately after injection contains radioactivity, but with time decreasing amounts are deposited so that the initial deposition is seen

Fig. 3



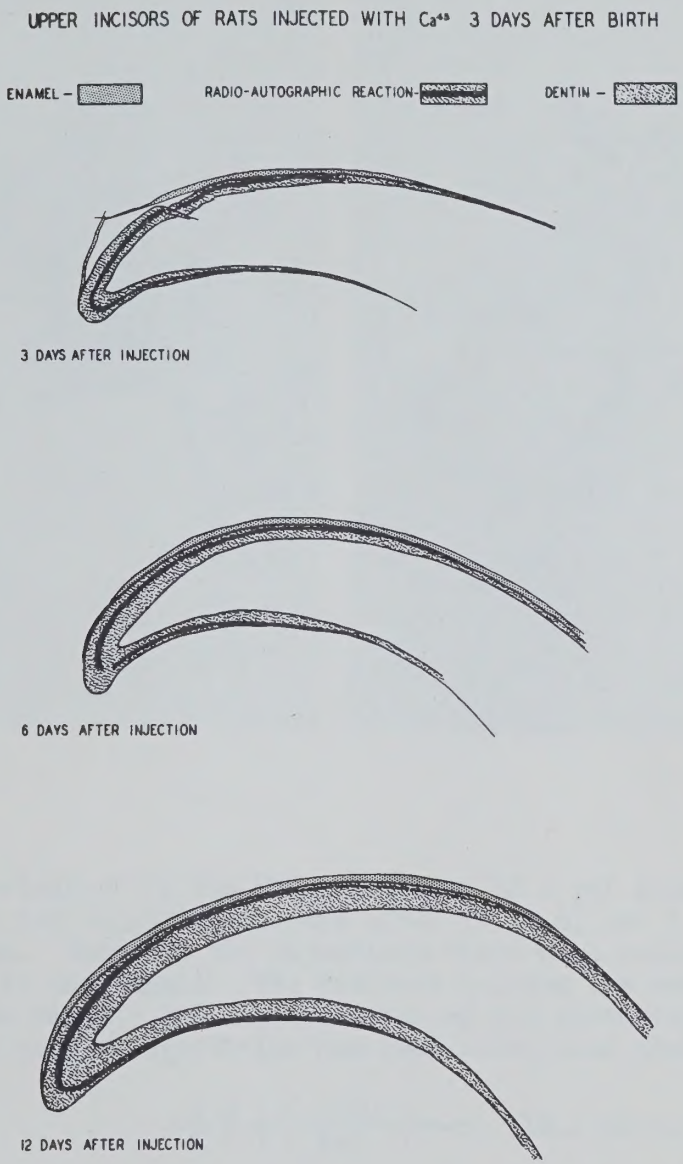
Ca^{45} radio-autograph of a lower incisor 8 days after injection to 3 day old rat. The band reaction is still present in the dentin. The enamel of this particular sample has been accidentally broken off.

Fig. 4



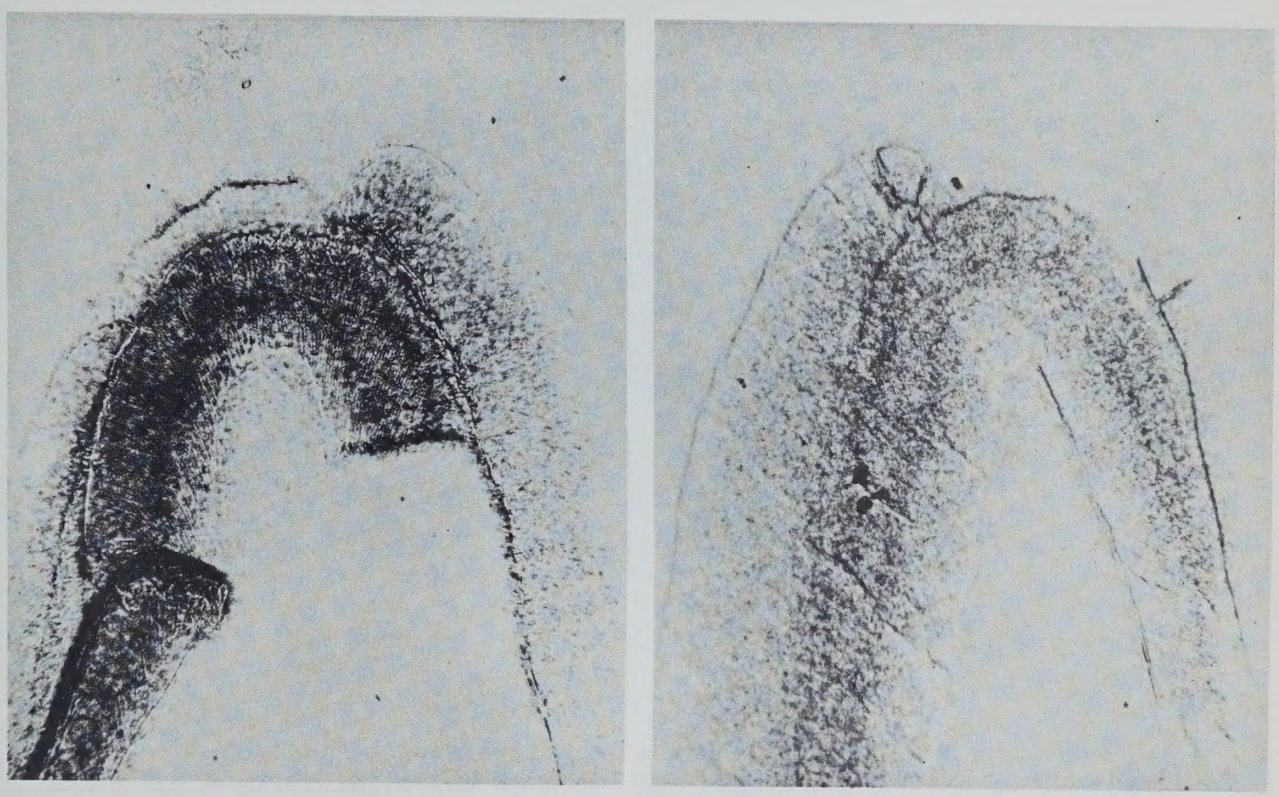
Ca^{45} radio-autograph of a molar of a rat injection at 3 days of age and sacrificed 9 days later. The band reaction although somewhat diffuse is still observed to be present in the dentin.

Fig. 5



Projection drawing of upper incisors of rats given Ca^{45} at 3 days of birth and sacrificed at 3, 6, and 12 days after injection. This drawing shows the relationships of the band reaction to the dentino-enamel junction.

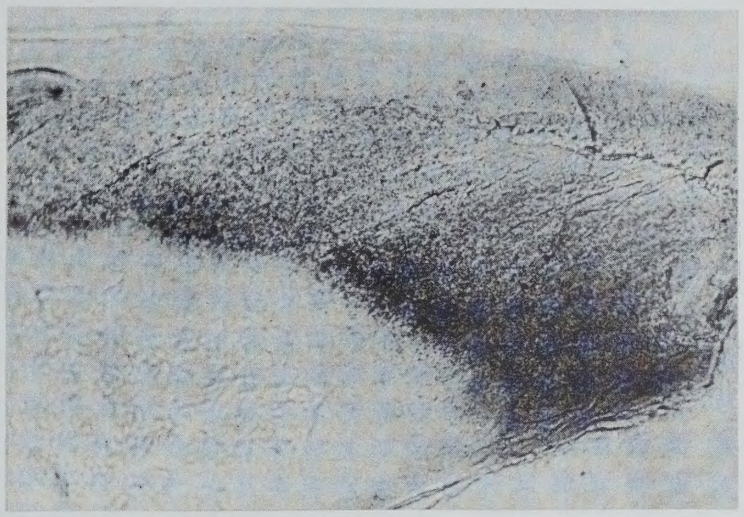
Figs. 6 and 7



Coated radio-autograph of the 3rd molar cusp of a rat injected with Ca^{45} at 3 and 6 days of age and sacrificed 3 days after (Fig. 6) and 5 days after (Fig. 7) the second injection. Note the two injections which were recorded separately in the dentin but not in the enamel. The distance between the bands in the tip of the cusp is 8 mm in both cases. The magnification of the photomicrograph is 145 x. Therefore the daily rate of deposition has been calculated thus:

$$\frac{\left(\frac{8}{3}\right)\left(\frac{1000}{145}\right)}{} = 18.4 \text{ micra per day.}$$

Fig. 8



Coated radio-autograph of a lower incisor of a 3 day old rat 3 hours after injection of P^{32}. The reaction obtained is identical in the dentin and enamel as in the Ca^{45} autograph. (Fig. 1).

APPENDIX "K"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Re: Project DR 23

by

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Radioautographic visualization of the formation and fate of the organic matrix of dentine

A radioautographic survey of the organs and tissues of very young rats injected with C^{14}-labelled bicarbonate revealed a widespread distribution of the radio-element.[1] The tissues of the developing incisors and molars displayed definite reactions, which were particularly intense in dentine. Evidence was presented to indicate that the sections of teeth used no longer contained minerals, even though they had not been systematically decalcified. It was, therefore, suggested that the radio-carbon had been incorporated into the dentinal matrix itself.[1]

A detailed study of this phenomenon was undertaken in the hope of extending our knowledge of the formation and fate of the organic component of dentine.

METHODS

The solutions of C^{14}-labelled bicarbonate used for injection were prepared from an alkaline solution of C^{14}-labelled sodium carbonate obtained from the Chalk River atomic pile. This material was neutralized by drop-wise addition of 4 per cent acetic acid up to the phenolphthalein end point (pH = 8.0) - a procedure which transforms most of the carbonate into bicarbonate. The volume of solution administered did not exceed 0.2 cc. and was injected as a single subcutaneous dose.

The first group of animals (hereafter referred to as the newborn group) consisted of the 10 newborn animals described in a previous paper.[1] It will be recalled that after receiving either 20 µc or 40 µc of the labelled bicarbonate, four of these animals were sacrificed four hours after injection, three at 24, and three at 72 hours after injection. For the present study, sections of alcohol-formol fixed jaws of these animals were decalcified by immersion in 5 per cent nitric acid for several minutes prior to being prepared for radioautography. The completeness of this decalcification procedure was assessed by means of the von Kossa technique for detection of calcium phosphate. While the dentine stains black by this method in the absence of nitric acid treatment, the lack of staining in

the treated sections was taken to indicate successful decalcification. Radioautography was carried out as indicated below.

A new group of 6 animals (hereafter referred to as the 3-day group) were three days of age at the time of administration of the C^{14}-labelled sodium bicarbonate. Three animals of this group received 30 µc of the labelled material and were then sacrificed at intervals of 1, 3, and 6 days after injection. The remaining 3 animals also received an initial injection of 30 µc, but in addition received a second injection of 30 µc two days after the first. These three animals were then sacrificed at 9, 12, and 15 days following the first of the two injections.

Maxillae and mandibles were removed from these animals immediately after sacrifice, and were then fixed in alcohol-formol[2], decalcified by the formate-citrate method[3], dehydrated in dioxane, embedded in paraffin, and sectioned at 5 µ.

The sections of teeth used for radioautography were either stained with hematoxylin-eosin or left unstained. Other sections were stained by the periodic acid-Schiff technique or by toluidine blue formetachromasia studies. In the latter case, a dilute solution of toluidine blue was used (0.1%). The preparations were not differentiated and were taken to balsam through acetone baths.

Radioautography was carried out by the coating technique[4] as more recently modified[5]. The emulsion utilized for this study was Eastman Kodak NTB3 emulsion, which was obtained either in the form of pellicles, to be melted before use, or in the form of a liquid which could be warmed and applied directly to the slides. Exposure times varied from 6 to 10 months at -17°C. The slides were subjected to routine photographic development and fixation, followed by dehydration in xylol, and mounting under Canada balsam.

The quantitative radioautographic technique of grain counting was applied in this study to determine the fate of labelled dentinal organic matrix. The rationale of this technique is based upon the fact that under conditions of low radioautographic exposure, the number of silver grains rendered developable by the ionizing radiations of C^{14} (or of any other Beta-emitting radio-isotope) is proportional to the amount of the C^{14} present in the source[6]. Thus, by counting the number of silver grains in the photographic emulsion lying over the tissue section in a developed radioautograph, it is possible to relate the count of the amount of isotope in the tissue.

Grain counting was carried out in the first lower molars of the animals of the 3-day group. The radioautographs used for this purpose were prepared under identical conditions throughout, i.e., all preparations were cut at the same thickness (7 µ), and were stained, coated, exposed and developed simultaneously. Moreover, the preparations were allowed only a short exposure in order to maintain the condition of proportionality of grain count to radio-activity.

The technique of counting grains was as follows. A reticle of two parallelly oriented hairs was inserted into one ocular of a binocular microscope. At the magnification generally utilized (approximately 800 diameters), the distance between the hairs in

relation to the specimen was of the order of 30 μ. The grain counts were obtained by enumerating all of the grains visible in successive strips of dentinal matrix measuring approximately 30 by 90 μ, beginning at the junction of the odontoblasts and predentine, and continuing to the dentino-enamel junction. After counting each strip, the slide under examination was moved in such a way that the next adjacent strip of matrix of the same dimensions was in the field provided by the reticle (Table 1).

In addition to these counts, several preliminary counts had been made on some of the sections utilizing an oil immersion objective which provided a total magnification of approximately 1200 diameters. At this magnification the distance between the two parallel hairs of the reticle in relation to the specimen was reduced to 20 μ, and the length of the strip was reduced correspondingly to approximately 60 μ. It was not possible to count all of the grains present in a given field under these conditions, since the depth of focus provided by the oil immersion objective was insufficient for this purpose. Hence, it was found necessary to count the grains in successive layers of the photographic emulsion for each field. This was accomplished by focussing on and counting those grains most nearly adjacent to the tissue. Following this, the barrel of the microscope was raised 2 μ by the fine adjustment and again those grains in focus were counted. Successive counts at 2 μ intervals were thus tallied and totalled. In this manner, the possibility of an error in counting was minimized and the total counts for each adjacent strip of dentinal matrix were obtained in a comparable fashion. All counts were made on the anterior cusp of the first lower molars of these animals, and were made in a strip perpendicular to the odontoblastic layer at the crown of the cusp (Fig. 9).

Some of the silver grains in the emulsion are not due to radiation, but are merely the background fog inherent in all photographic emulsions. To assess the extent of such background grains, several successive counts were made of grains lying in the photographic emulsion in areas devoid of tissue. These "background" counts were averaged, and were then subtracted from the counts obtained from the tissues.

RESULTS

Morphological observations

A_2 The dentinal organ consists of the pulp, the odontoblasts, the predentine and the dentine itself, which ends at the "junction" that is, according to the area, dentine-enamel or dentino-cemental junction. Predentine stains poorly and dentine deeply with hematoxylin eosin (Fig. 1). The dentine was also intensely metachromatic and the toluidin blue technique for metachromasia (Fig. 16) and the periodic acid-Schiff technique (Fig. 17). Radioautographic observations follow.

B_2 In the newborn group, the incisor and molar teeth at four hours after injection showed a precisely localized and relatively intense radioautographic reaction over the region of predentine immediately adjacent to the odontoblasts (Fig. 1). No reaction was observed over the dentine already present in the teeth at this interval (note unreactive dentine (D) in Fig. 1). The latter

observation was further emphasized by the fact that the reaction of predentine was clearly visualized even in areas where no dentine had yet formed (Fig. 2). Little or no reaction was observed over the odontoblasts themselves.

By 24 hours, little change in the intensity of this reaction was observed, but its distribution was slightly altered. Some reaction was present in predentine, while most of it was in that portion of dentine in apposition with the predentine. In some of the areas where only predentine had been present at four hours, some reactive dentine had now appeared.

At 72 hours, the predentine was unreactive, while many silver grains were seen over the dentine proper. No odontoblastic reaction was seen at this or any later time interval.

It may therefore be concluded that the reactive material was present exclusively in the predentine at four hours and in the dentine at 72 hours after injection.

The radioautographic reactions of teeth seen in the three-day group of animals at the early time intervals presented essentially the same features as did those of the newborn group. At 24 hours after injection, the most intense radioautographic images in the molars occupied a position similar to that seen in the newborn group at the same interval, that is, in the inner layers of dentine and in the juxtaposer outer layers of predentine (Fig. 10). This reaction extended throughout the complete circumference of the molar bud (Fig. 3). Since the area of predentine immediately in contact with the odontoblasts contained very few granules (Fig. 10), the predentine laid down during the latter part of the 24-hour period between C^{14} injection and sacrifice contained only a small amount of radioactivity. In other words, most of the labelled material had been deposited during the earlier part of this 24-hour period.

By three days after injection, the band of reaction was strictly limited to the dentine where it was seen as a definite and discrete line about midway between the odontoblasts and the dentino-enamel junction (Fig. 11). Comparison of this pattern with that seen at 24 hours indicated that the distance between the line of radio-autographic reaction and the dentino-enamel junction had not changed over the intervening 48-hour period, that is, an area of unreactive dentine of the same approximate thickness was observed between the reactive line and the dentino-enamel junction at both 24 and 72 hours. Since most of the labelled material was deposited during the earlier part of the 24-hour period following C^{14} injection, a preparation such as that depicted in Figure 4 may be interpreted with precision according to whether the dentine is present outside or inside the dark band (Fig. 4), it was laid down respectively before or after the earlier part of the 24-hour period following C^{14} injection.

By six days after injection (Fig. 12), the band of autographic reaction in the dentine remained clearcut and its intensity appeared the same as at the earlier intervals. The distance between the line and the dentino-enamel junction on the cusp crowns also remained approximately the same as that seen at the earlier times. More new

dentine had been formed by the odontoblastic layer, however, so the distance between the line and the epithelium had increased further. The reaction line followed the contours of the cusps (Fig. 5) lying at some distance from the dentino-enamel junction at the crowns, but lying against the junction at the bases of the cusps. By this time, the roots of the molars had lengthened, and naturally the new dentine deposited near the tips of the roots was unlabelled.

The animal sacrificed at nine days after injection had received a second injection of C^{14}-labelled bicarbonate two days after the first, and indeed the radioautographs showed a double line of reaction in the dentine (Fig. 13). Again, the intensity of each of these lines was similar to that of the lines seen at the earlier intervals. The outer and inner lines, resulting from the first and second injection respectively, were separated by a layer of unreactive dentine which was clearly seen only in the region of the crowns of the cusps. In the area of the roots, the lines were fused (Fig. 6).

The radioautographic images in the molars of the doubly-injected animal sacrificed at 12 days were essentially the same as the nine-day animal (Figs. 7 and 14).

In the animal sacrificed at 15 days, the molar teeth had erupted. The distinction between two reaction lines was not clear in this case (Figs. 8 and 15). The distance between the line and the dentino-enamel junction was approximately the same here (Fig. 8) as in the animals sacrificed at 1, 3,and 6 days (Figs. 3-5), but was somewhat greater than at 9 and 12 days (Figs. 6 and 7), possibly due to individual variations.

The reactions of incisor teeth in the three-day group of animals were analogous to those just described in the molars, within the limits of comparison of the two types of teeth.

Grain Counting

To confirm the observation that there seemed to be little or no change in the intensity of the autographic reactions visualized in the dentinal matrix of molars with time after injection, it was decided to make a quantitative study of the autographic reactions in the molars of the three-day group.

To show the feasibility of the method, the silver grains were counted over strips of dentinal matrix at the anterior cusp crown of the first lower molar at a magnification of 1200 diameters (oil immersion). The result obtained in a 9-day animal are illustrated in fig. 9, in which the peaks in the graph of the counts correspond to the two bands of reaction seen in the photograph below it.

The thickness of some of the other preparations made it impossible to examine them all at 1200 diameters. Systematic counts were, therefore, carried out at a magnification of 800 diameters, utilizing radioautographic preparations from the animals sacrificed at 3, 6, 9, 12 and 15 days. All of the

silver grains present over strips of dentinal matrix on the anterior cusp crown of the first lower molar from each animal were counted. From these counts, the "background" count was subtracted to yield the data presented in Table 1. (The "background" values obtained were respectively: 3 days, 66; 6 days, 64; 9 days, 56; 12 days, 70; and 15 days, 79).

The results obtained at 800 diameters did not make it possible to distinguish the two peaks seen at 1200 diameters (Fig. 9). In order to obtain a measure of the intensity of the reaction lines seen at the various intervals, the two greatest values were noted and totalled for each animal which had received but one injection of the isotope. In the case of the doubly-injected animals, the four highest values were used for this purpose. These values, probably the best ones to use for comparative purposes, are underlined in the body of Table 1, and their total is also underlined as the "peak count total".

Examination of Table 1 reveals that whether the "total count" or the "peak count total" is considered, the quantity of silver grains deposited in the photographic emulsion of the radioautographs was approximately the same in the animals given a single injection (3 and 6 days) and about double that value in the animals given two injections (9, 12, and 15 days). It is clear that there was no decrease with time in the amount of labelled material deposited in the matrix.

DISCUSSION

The presence of radioautographic reactions in the matrix of the dentine in growing rats injected with C^{14} bicarbonate (Figs. 10-15) is a finding of significance in regard to the growth pattern, the composition, and the role of the matrix, in tooth development.

Growth pattern of dentinal matrix

As early as 4 hours after injection of labelled bicarbonate, radioactivity appeared in the portion of the predentine juxtaposed to the apices of the odontoblasts (Figs. 1 and 2).

The radioactive band seen in the sections was due to the presence of a layer of C^{14} labelled predentine matrix located near the odontoblasts. Apparently, some of the injected C^{14} had been utilized for the elaboration of the predentine matrix deposited during this 4-hour period.

At 24 hours after injection, the layer of labelled matrix had been displaced outward due to the continuing apposition of new, unlabelled layers of predentine. Furthermore, the labelled layer-initially predentinal was changing into dentinal matrix (Fig. 10).

At 3 days, and at later times after the injection of labelled bicarbonate, the growth of dentine was visualized by a progressive increase in the distance between the initial labelled band and the odontoblasts (Figs. 10-15). This distance increased very rapidly at first (Figs. 10-12), more slowly at the longer time intervals (Figs. 13-15). Thus, although these conclusions are based on only 6 animals, it appears that the growth rate of dentinal

matrix was rapid in animals during the first 10 days of life alter which it slowed down.

Examination of the growth of the various regions of the molar tooth (Figs. 3-8) illustrated several points already observed by other methods[7,8], namely, the continued root growth, the progressive encroachment of dentine into the pulp cavity - a process balanced by the expansion of this cavity into the root region and in doubly injected rats the more rapid apposition of dentinal matrix in the cusps of the crown than in the roots, as shown by the distinctness of the two lines of reaction in the former but not in the latter area (Figs. 6 and 7).

Two properties of the dentinal matrix may be emphasized as a result of this work, namely, its incremental formation and its stability. The doubly injected animals clearly show the incremental deposition of matrix (Figs. 6 and 7). In addition, the absence of silver grains in the area between the reactive band and the dentino-enamel junction indicates that no interstitial deposition of material occurs in formed matrix. The presence of silver grains in the matrix laid down for some time after injection (Table 1) does not disprove that dentine grows incrementally since the grains are few and presumably indicates decreasing blood lines of the C^{14} utilizable for matrix formation.

The dentinal matrix -- as indicated by its labelled portion -- is a stable substance. The grain counts through the 15 days after injection showed no decrease in the amount of labelled material (Table 1). Such stability of the matrix is all the more impressive since a survey of the distribution of C^{14} bicarbonate in very young rat tissues showed a generalized and almost complete decrease in the radioautographic itensity of organs and tissues with time - a fact attributed to turnover of structural components[1]. Thus the dentinal matrix stands out as one of the most stable tissues of the body.

Mineral elements of dentine also are formed incrementally as shown by P^{32} radioautography[9] and other methods[7,8]. However, the exactness of their incremental deposition as shown by either P^{32} or Ca^{45} radioautography is less striking. In fact, some interstitial deposition if minerals throughout the thickness of the dentine, even acclusally to the main band of reaction, is usual in young animals. Newborns will often show no definable band at all but rather a diffuse reaction. In addition, a reaction band often becomes less precise with time[2], although it always persists in some form[10]. It is indeed believed that mechanical influences may produce a re-arrangement of dentinal crystals[11]. Therefore, it is concluded that the matrix is deposited in a regular manner and is more stable than the mineral components of dentine.

Nature of the labelled substances in the dentinal matrix

It has been pointed out that the labelled substances detected by radioautography in organs and tissues from animals given C^{14} bicarbonate were likely to be substances of large molecular weight, such as proteins or polysaccharides[1]. This is probably true of the labelled component(s) of the dentinal matrix. It is known that this matrix consists of a protein framework, presumably a collagen, associated with mucopolysaccharide(s) which were detected by the metachromatic

staining of the matrix with toluidin blue and by its reactivity to the periodic acid-Schiff technique.[12,13,14] In contrast, the predentinal matrix failed to stain or stained only slightly when the latter two techniques were used, as shown in Figs. 16 and 17. Thus, the predentine appeared to consist of collagenous material only.[14] Deposition of mucopolysaccharidic material presumably occurred at the predentino-dentinal junction. In fact, the presence or absence of mucopolysaccharide(s) could account for the observed differences between the predentine and dentine.[1]

If this is the case, the labelled material appearing in predentine after C^{14}-bicarbonate injection would be part of the collagen framework. Since the radioautographs showed no apparent change in this labelled material at the time of the transformation of predentine into dentine, the collagen of the first zone would not differ from that of the other zone except for the addition of mucopolysaccharide(s).

It is known that calcium phosphate crystals are absent in predentine and abundant in dentine, and also that radioautographs show the deposition of Ca^{45} and P^{32} in that area of the dentine which is in apposition with predentine[10], that is, where mucopolysaccharide(s) also appear. It is tempting to conclude that the mucopolysaccharide(s) may be necessary for the deposition of the dentine crystals and may, in fact, bind these crystals to the collagen framework.[2]

SUMMARY

Sodium bicarbonate labelled with C^{14} was administered to newborn and three-day old albino rats, whereupon these animals were sacrificed at intervals of from four hours to 15 days after injection. Radioautographs of their teeth were prepared after decalcification.

The radio-carbon of bicarbonate is incorporated into the organic matrix of the dentinal organ. At the earliest time intervals studied, the labelled matrix lies in the predentinal area. But at later intervals the reactive zone is farther and farther away from the odontoblasts as new, non-labelled predentinal matrix is laid down. In the process, the labelled matrix becomes dentine.

Counts of the individual silver grains constituting the radioautographic image of the matrix show no decrease in its intensity during the 15-day periods studied. Thus, the labelled matrix and, by inference, the whole matrix of dentine is a very stable structure (in contrast to C^{14}-labelled structures formed in other organs).

The formation of dentine may be considered to occur in three steps: 1) the formation of a matrix framework -- presumably a collagen -- which first appears as predentine at the apices of the odontoblasts. (This collagenous material would be the substance which is traced by means of C^{14});2)the addition of mucopolysaccharide(s) to the framework of the matrix -- changing it from predentine to dentine;and 3) the appearance of calcium phosphate crystals in the matrix of dentine.

While the mucopolysaccharide(s) and crystals are acquired simultaneously at the predentino-dentinal junction, it is here suggested that the crystals are bound to the collagen matrix of dentine by means of the mucopolysaccharide(s).

ACKNOWLEDGEMENT

This work was supported by a grant of the Associate Committee on Dental Research of the National Research Council of Canada.

FOOTNOTES

1. The presence of mucopolysaccharides in predentine has been suggested by Verne et al[15,16] on the basis of evidence contrary to that presented here.

While the discrepancy cannot be explained at this time, an alternative explanation of our findings might be that mucopolysaccharide(s) possessing no groups reactive to the metachromasia and periodic acid-Schiff techniques might exist in predentine. Hence, histochemically reactive groups would have to be acquired at the time of the transformation of predentine into dentine. However, from the chemical point of view, it is difficult to visualize the simultaneous occurrence of:

1) the removal of a radical blocking the 1,2 glycol, or similar group(s), necessary for periodic acid-Schiff reactivity and

2) the addition of a sulfonic, or other acid group, necessary for metachromatic staining.

2. In the case where only the histochemically reactive groups of mucopolysaccharides were to appear at the predentino-dentinal junction, such groups would have to fulfil the role of holding the crystals of calcium phosphate to the collagen of the matrix.

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LEGEND OF THE FIGURES

FIG. 1: Coated radioautograph of a portion of an incisor tooth taken from a rat injected at birth with 4 μc C^{14}-bicarbonate and sacrificed 4 hours later. The section was stained with hematoxylin and iosin prior to coating with Eastman-Kodak NTB3 emulsion. X180

A: ameloblasts; PE: pre-enamel; D: dentine; PD: predentine; O: odontoblasts.

The dentine which appears here as a dark layer shows no significant radioautographic reaction. The predentine, which appears light, shows a radioautographic reaction in the area close to the tips of the odontoblasts. (A slight reaction is present in pre-enamel).

FIG. 2: Coated radioautograph of the root region of an incisor tooth taken from the same animal as in Fig. 1, but the preparation was exposed for a longer time. The abbreviations are the same as in Fig. 1.

In this area no dentine has yet formed. Even so, the predentine shows an intense reaction. On the left of the figure the predentine had broken away from the pre-enamel during the technical procedure. This artifact makes it all the more evident that the reaction is in predentine.

FIGS. 3-8: Diagrams of coated radioautographs of the first molars taken from rats injected with C^{14}-bicarbonate when 3-day old and sacrificed at the following intervals after injection: 1 day (Fig. 3); 3 days, (Fig. 4); 6 days (Fig. 5); 9 days (Fig. 6); 12 days (Fig. 7); 15 days (Fig. 8). In addition, the animals sacrificed at the 3 last intervals received a second injection of C^{14}-bicarbonate when 6-day old.

FIG. 9: Grain count in the dentine from the region of the odontoblasts to the junction in an animal injected twice with C^{14}-bicarbonate, namely, at 3 and 6 days after birth. The animal was sacrificed 9 days after the first injection. The silver grains were counted at a magnification of 1200 diameters.

The two peaks seen in the graph correspond to the two bands of reaction seen in the dentine.

FIGS. 10-15: High magnification of the first cusp of the crown of the first molar from the animals of the 3-day group. After injection of C^{14}-bicarbonate when 3-day old, they were sacrificed at: 1 day (Fig. 10); 3 days (Fig. 11); 6 days (Fig. 12); 9 days (Fig. 13); 12 days (Fig. 14); 15 days (Fig. 15). (The last three animals received a second injection three days after the first). O: odontoblasts; D: dentine; J: dentino-enamel junction.

FIG. 16: Toluidine blue staining of molar tooth of 3-day old rat. Abbreviations as in Fig. 1.

LEGEND OF THE FIGURES (Cont'd)

The nuclei of the ameloblasts and odontoblasts stain blue. The dentine is a deep pink and the predentine a very light pink.

FIG. 17: Periodic Acid -- Schiff staining of molar tooth of 3-day old rat. Counterstaining with hematoxylin. (This section was taken from the center of the cusp, and, therefore, shows at the apex an area devoid of enamel).

The dentine stains deeply and the predentine little or not with the periodic acid-Schiff technique.

TABLE I

GRAIN COUNT OF ANTERIOR CUSP OF FIRST LOWER MOLAR

(Peak count underlined)

Distance in μ from Odontoblasts	Animal Sacrificed at 3 Days (fig.5)	6 Days (Fig.6)	9 Days (fig.7)	12 Days (fig.8)	15 Days (fig.9)
0-30	58	16	14	9	5
30-60	171	34	36	24	47
60-90	392	45	57	61	22
90-120	40	119	71	42	30
120-150	18	393	152	140	86
150-180		81	186	337	76
180-210			233	463	94
210-240			467	409	82
240-270			74	113	155
270-300					230
300-330					393
330-360					477
360-390					65
390-420					16
420-450					8
	t___	t___	t___	t___	t___
Total Count	679	688	1290	1598	1786
Peak Count Total	563	512	1038	1349	1255

TABLE I

GRAIN COUNT OF ANTERIOR CUSP OF FIRST LOWER MOLAR

(Peak count underlined)

Distance in μ from Odontoblasts	Animal Sacrificed 9 Days (Fig.5)	12 Days (Fig.6)	15 Days (Fig.7)	18 Days (Fig.8)	21 Days (Fig.9)
0-30	58	16	14	9	5
30-60	171	34	36	24	47
60-90	392	45	57	61	22
90-120	40	119	71	42	30
120-150	18	193	152	140	86
150-180		81	186	137	76
180-210			233	103	94
210-240			107	109	82
240-270			74	113	135
270-300					230
300-330					303
330-360					177
360-390					65
390-420					16
420-450					8
	±	±	±	±	±
Total Count	679	688	1290	1598	1786
Peak Count Total	563	512	1038	1349	1255

Fig. 1

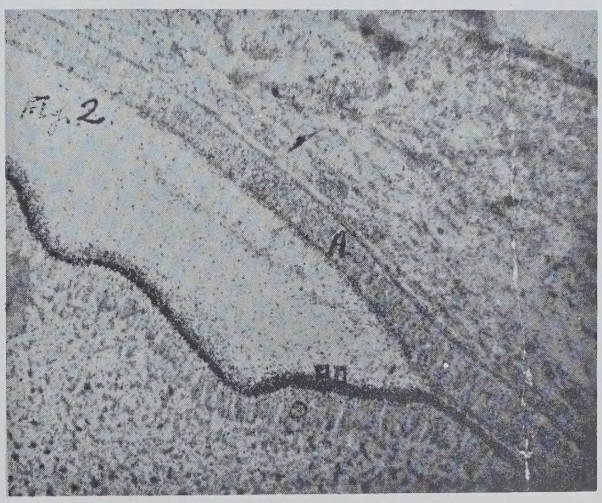
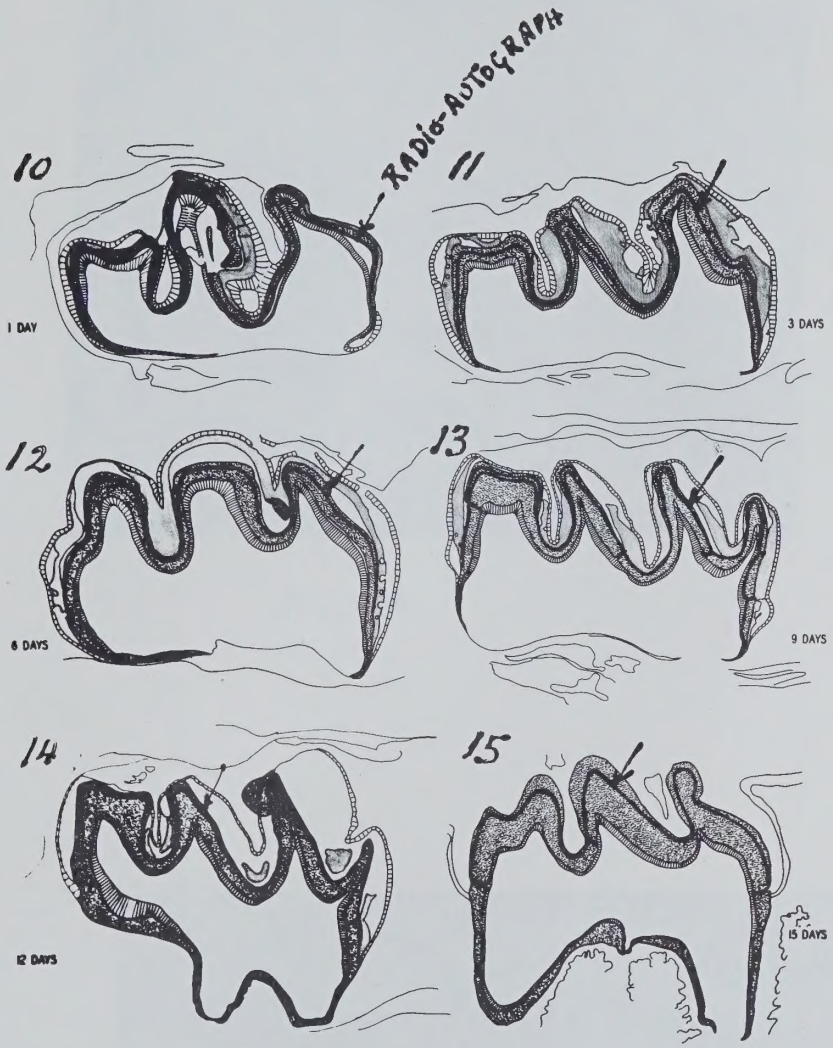


Fig. 2


RADIO-AUTOGRAPH
10
1 DAY
11
3 DAYS
12
6 DAYS
13
9 DAYS
14
12 DAYS
15
15 DAYS

Figs. 10-15

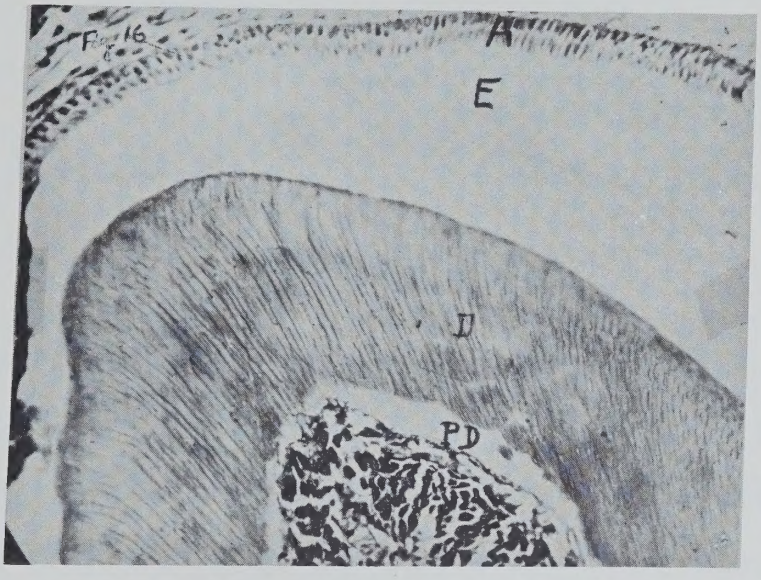


Fig. 16

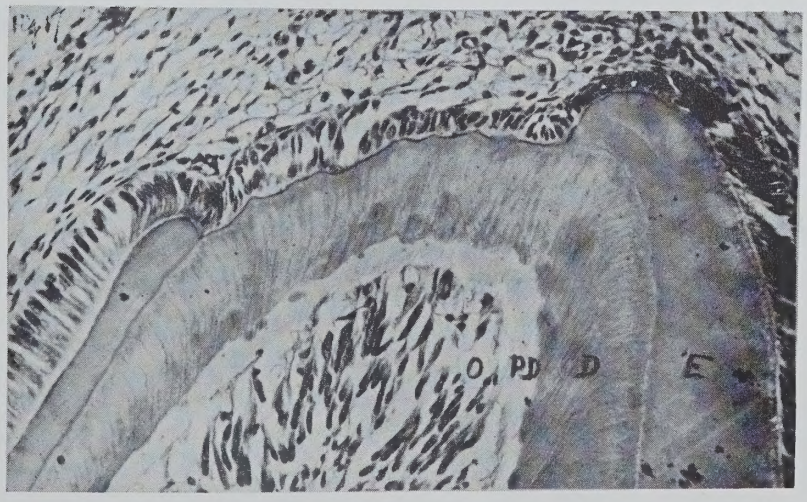


Fig. 17

APPENDIX "L"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: Investigation of methods for destroying the organic matter in teeth, bones and other materials prior to determination of fluorine content.

Grantee: Dr. O. J. Walker, Department of Chemistry, University of Alberta.

Project No.: DR 28 Amount of Grant: $1,000

SUMMARY OF WORK

Experiments carried out on this project in 1952-53 were repeated by the research assistant, Miss Hinda Doz, and additional ashing agents were studied. Some difficulty has been met with in the choice of satisfactory ashing agents. Best results were obtained with sodium hydroxide, potassium hydroxide, sodium peroxide and mixtures of potassium hydroxide and magnesium nitrate. All of these gave high results for 0.00 to 0.05 mg. of fluoride but satisfactory recovery for 0.05 to 0.20 mg.

All experiments carried out so far have dealt with the use of starch as the organic material. When more positive results are obtained it is planned to try the method on teeth.

Experiments carried out indicate that the distillation step in the procedure is fairly satisfactory.

APPENDIX "I"

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Project No.: DR 28 Amount of Grant: $1,000

Interim Report

The successful ashing of organic material containing known amounts of fluoride using alkaline ashing materials has been carried out. Range of satisfactory fluoride recovery was 0.10 to 0.25 mgs.

Ashings were carried out on fluoride free material to which known amounts of fluoride in the form of sodium fluoride (1) was added.

One gram samples of starch with the appropriate ashing material and fluoride content were heated in nickel crucibles under the low heat of a hot plate until all liquid had evaporated. More heat was then applied until carbonisation was complete. Ashing was carried out in an electric furnace at 600-700°C. for approximately one hour or until ashing had reached completion.

The ashed product was then transferred to a pyrex flask to which 110 ml. of distilled water and 40 ml. of concentrated sulfuric acid was added. The acid was poured in very carefully and only after the flask was connected to the condenser was the mixture shaken. Method of distillation was a modified Thrun (2) procedure.

Distillation was from "all glass" apparatus using a 51 cm. condenser. Between 89 and 90 ml. of distillate were collected and made up to a volume of 100 ml. If more than 90 ml. of distillate was collected sulfate ion was then present in quantities large enough to affect fluoride recovery. Time of distillation was 8-12 minutes. Loss of vapor was controlled by first heating the flask under low heat of a Meker burner. Heat was turned up after a few milliliters of distillate had been collected. It was impossible to complete a distillation under 15 minutes if Bunsen burners were used. Bumping was controlled by thorough shaking prior to distillation and by

the presence of a number of glass beads. Use of silver sulfate to prevent volatilization of fluoride (2) was discontinued after a series of distillations showed that this reagent had very little effect.

Fluoride concentration was determined by using the sodium alizarin sulfonate photoelectric colorimeter method as outlined by O. J. Walker et al. (3). Sulfate which interfered if more than 300 p.p.m. was present was determined by the T.H.Q. (tetrahydroquinone) method (4).

The following substances were used as ashing reagents: potassium hydroxide, sodium hydroxide, sodium peroxide, magnesium nitrate, calcium hydroxide and barium peroxide.

a. Sodium hydroxide, potassium hydroxide and sodium peroxide

Results obtained using potassium hydroxide, sodium hydroxide, and sodium peroxide were very similar. One gram samples of sodium hydroxide and potassium hydroxide were used whereas only one-half gram of sodium peroxide was used due to the violent reaction between the peroxide and the fluoride solution. In the lower ranges 0.00-0.05 mg. fluoride yields were erratic and very large. At present it is impossible to account for the discrepancies and it can only be suggested that improvements in the distillation technique be devised. The 0.05-0.20 mg. range is very favorable. No exhaustive study has been carried out beyond this range but recovery of up to 0.50 mg. fluoride appears possible. The following figures are representative of the results obtained from eight series of determinations:

mg. F. added	mg. F. recovered
0.00	0.08
0.05	0.08
0.10	0.12
0.15	0.15
0.20	0.18

In adding known amounts of fluoride to the distillation flask and then determining the efficiency of the distillation technique yields were found to be usually 0.01 mg. higher than the added amount. Hence the results obtained from the various ashings were within experimental error.

b. Mixture of one gm. potassium hydroxide and one ml. 20% magnesium nitrate

In using a mixture of one gm. of potassium hydroxide and 1 ml. of 20% magnesium hydroxide solution as ashing reagent favorable results between 0.15 and 0.25 mg. fluoride were obtained.

mg. F. added	mg. F. recovered
0.00	0.10
0.05	0.13
0.10	0.16
0.15	0.18
0.20	0.22
0.25	0.26
0.30	0.34

These are the averages of four series.

High yields are again unaccountable in the lower range.

c. Magnesium Nitrate

One ml. of a 20% magnesium nitrate solution was used as ashing reagent. Results obtained were extremely low. pH measurements indicated that the solution was acid and hence it was thought that volatilization of fluoride may have occurred in the preliminary evaporation since magnesium nitrate is a salt of the weak base, strong acid type.

Though unsatisfactory, the oxidizing powers of magnesium nitrate should not be overlooked since this represents a powerful means of breaking up organic material. It is suggested that investigation be made regarding the possibility of obtaining mixture of magnesium nitrate with reagents which will counteract its acidic nature.

d. Calcium Hydroxide

Calcium hydroxide was used as ashing reagent in quantities varying from one to five grams. Results obtained were extremely erratic with yields varying from 0-1400%.

Ash residue consisted of calcium oxide and calcium fluoride. Since calcium oxide is insoluble in sulfuric acid it was necessary to filter prior to distillation. It is thought that low yields resulted from incomplete solution of the calcium fluoride. It was believed that the calcium fluoride might be reclaimed treating with dilute sulfuric acid and then filtering. However, sulfate concentration was then extremely high and caused interference in fluoride determinations.

Samples were determined in which the calcium oxide was not filtered off. Results were extremely high in these uses. It is suggested that Ca^{++} ion, which may have been coming over in the distillate, had an effect on the indicator. Since the indicator is a colloid the ion may have been adsorbed on the surface, hence leaving less area to be bleached by the fluoride ion. Bleaching would then be greater on the remaining surface area and therefore fluoride recovery would be higher.

e. Barium Peroxide

One gram of BaO_2 was used as an ashing reagent. Recovery of fluoride was very low. Here again the problem of an insoluble component is encountered. There is thus the possibility of co-precipitation of the barium fluoride. This may account for the low yield of fluoride.

Conclusion

Results from alkaline ashing are more favorable when soluble ashing reagents are used. Until further experiments are carried out on insoluble ash residues it is suggested that reagents such as these are unsatisfactory for ashing. A difficulty which must still be overcome is the development of a technique for the recovery of small amounts of fluoride i.e. 0.00-0.05 mg. Also a vast source of ashing reagent which should be looked into is the possibility of mixtures of oxidizing agents and strong bases.

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APPENDIX "L"

Discussion on Project DR 28

Dr. J.M.R. Beveridge submitted the following memorandum to the Secretary of the Committee in support of his observations as recorded in Min. 10 of these proceedings (18th Mtg.).

The statement that satisfactory recovery for amounts of fluoride between 0.05 and 0.20 mg. fluoride gives the impression that a valid procedure for the determination of fluoride in organic material has been devised. However, the analytical data cannot be taken as supporting this since no account was taken of the quite considerable blank equivalent to 0.08 - 0.10 mg. fluoride. When the results are corrected for the blank the actual recoveries vary from zero to as much as about 83%. The grantee states that "high yields are again unaccountable in the lower range." These high results are of course readily accounted for on the basis of the relatively large blank determination for which no correction has been made in the recovery figures.

If an adequate distilling head were used during the distillation step it is difficult to understand why sulphate should appear in the distillate. The production of 90 ml. distillate in as short a time as 8 minutes would appear to be an extremely rapid rate of distillation for the usual type of laboratory still and the possibility is raised that a fine spray of sulphuric acid may be carried over due to the rapidity of the distillation and relative ineffectiveness of the distilling head.

APPENDIX "M"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: Fluoride content of dentine, enamel and alveolar processes of teeth for Sarnia, Brantford and Stratford.

Grantee: M. Doreen Smith, Department of Food Chemistry, University of Toronto

Project No.: DR 13 Amount of Grant: $1625

The separation of enamel and dentine of teeth and the subsequent analysis of these fractions for fluorine has been in operation since June 1953, on teeth which have come from Brantford, Ontario. No additional teeth from Sarnia or Stratford have been received. However, a reasonable number have been analyzed from these areas and it is expected that the picture, with respect to the fluoride content, will be more or less static. In the case of the Brantford teeth the interest lies in following the fluoride content in relation to the time of exposure to the fluoridated water.

The fluorine figures in Tables I and II are from 39 teeth extracted within the period May 1952-May 1953. Fluoridation of Brantford water was commenced early in 1945 so that subjects from whom these teeth were extracted were exposed to fluoridated water for approximately seven years. The age range of the subjects was from 5 years to 17 years.

From Table III which shows averages, it can be seen that the average analysis for the teeth from the Brantford area after 1951, are higher than for the teeth extracted up to 1948, and their fluoride composition is closer to those from the Stratford area than from the Sarnia area.

TABLE I

FLUORINE CONTENT OF DECIDUOUS TEETH FROM BRANTFORD

No.	Age	Sex	Type of Tooth	Degree of Caries	% Fluorine in Dentine	% Fluorine in Enamel	D/E
1.	6	M	Molar	Very severe	.0252	.0109	2.3
2.	7	M	Molar	Very severe	.0127	.0064	2.0
3.	8	F	Molar	Severe	.0065	.0071	.9
4.	8	F	Molar	Slight	.0199	.0044	4.5
5.	9	F	Molar	Severe	.0426	.0087	4.9
6.	12	F	Molar	Severe	.0227	.0034	6.7
				Average	.0216	.0068	3.6

TABLE II

FLUORINE CONTENT OF BRANTFORD TEETH

No.	Age	Sex	Type of Tooth	Degree of Caries	% Fluorine in Dentine	% Fluorine in Enamel	D/E
1.	5	M	Molar	Moderate	.0478	.0186	2.6
2.	6	M	Molar	Moderate	.0343	.0153	2.2
3.	7	M	Molar	Severe	.0228	.0084	2.7
4.	8	F	Molar	Moderate	.0328	.0073	4.5
5.	9	F	Molar	Moderate	.0292	.0104	2.8
6.	9	F	Bicuspid	Severe	.0267	.0093	2.9
7.	9	F	Molar	Severe	.0487	.0182	2.7
8.	9	F	Molar	Severe	.0468	.0214	2.2
9.	9	F	Molar	Severe	.0474	.0178	2.7
10.	11	F	Bicuspid	None	.0383	.0096	4.0
11.	11	F	Bicuspid	Moderate	.0891	.0265	3.4
12.	11	F	Molar	Severe	.0473	.0095	5.0
13.	11	F	Bicuspid	None	.0338	.0130	2.6
14.	11	F	Molar	None	.0339	.0341	1.0
15.	11	M	Bicuspid	None	.0480	.0128	3.8
16.	11	F	Bicuspid	None	.0182	.0101	1.8
17.	11	F	Molar	Severe	.0348	.0038	9.2
18.	11	M	Incisor	None	.0279	.0054	5.2
19.	11	F	Bicuspid	None	.0357	.0138	2.6
20.	13	M	Bicuspid	None	.0396	.0116	3.4
21.	13	F	Molar	Very severe	.0390	.0073	5.3
22.	13	F	Bicuspid	Severe	.0384	.0110	3.5
23.	13	F	Molar	Very severe	.0395	.0080	4.9
24.	14	F	Molar	Moderate	.0165	.0078	2.1
25.	14	F	Molar	Severe	.0486	.0130	3.7
26.	14	M	Molar	Severe	.0335	.0578	.6
27.	14	M	Molar	Very severe	.0229	.0079	2.9
28.	15	M	Molar	Very severe	.0225	.0235	1.0
29.	15	F	Bicuspid	None	.0439		
30.	15	F	Bicuspid	None	.0479	.0118	4.1
31.	15	F	Bicuspid	Very severe	.0360	.0068	5.3
32.	16	M	Molar	Severe	.0406	.0116	3.5
33.	17	F	Molar	Moderate	.0518	.0083	6.2
				Average	.0383	.0141	3.45

TABLE III

AVERAGES, INCLUDING SARNIA, BRANTFORD AND STRATFORD

No. of teeth	Area of Extraction	Dentine content %Fluorine	Enamel content % Fluorine	D/E
24	Sarnia	0.0104	0.0048	2.7
15	Brantford, prior to '48	0.0167	0.0024	2.7
33	Brantford, '52-'53	0.0383	0.0141	3.5
40	Stratford	0.0559	0.0222	3.5

APPENDIX "N"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: The effect of the endocrines on the teeth and jaws. II. The effect of propyl thiouracil on the bone of the jaws of rats.

Grantee: K.J. Paynter, Department of Oral Anatomy, Division of Dental Research, University of Toronto

Project No.: DR 38 Amount of Grant: $1000

SUMMARY OF WORK

1. The effect of propyl-thiouracil-induced hypothyroidism on the development of molar teeth in rats has been studied, beginning at the time of initiation of the Lamina for these teeth.

2. Histological evidence indicates that the teeth develop in a normal manner even though the animals are suffering from a deficiency of Thyroid hormone.

3. At the older ages (30-36 days) it appeared that tooth eruption became retarded more than other processes involved in tooth formation, and may have ceased altogether.

4. It is proposed to change the name of the project to read:

The Effect of the Endocrines on the Teeth and Jaws. II. Further Studies on the Effect of Thiouracil on the Development of Molar Teeth of Rats.

5. It is felt that one more report should bring this project to a conclusion, and that remaining work on it can be done without spending more money. It is therefore proposed that the grant be suspended at this date.

APPENDIX "A"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: The effect of the endocrines on the teeth and jaws.

Grantee: K.L. Paynter, Department of Oral Anatomy,
Division of Dental Research,
University of Toronto

Project No.: DR 35 Amount of Grant: $1000

SUMMARY OF WORK

1. The effect of propyl-thiouracil-induced hypothyroidism on the development of molar teeth in rats has been studied, beginning at the time of initiation of the lamina for these teeth.

2. Histological evidence indicates that the teeth develop in a normal manner, even though the animals are suffering from a deficiency of Thyroid hormone.

3. At the older ages (30-36 days) it appeared that tooth eruption became retarded more than other processes involved in tooth formation, and may have ceased altogether.

4. It is proposed to change the name of the project to read:

The Effect of the Endocrines on the Teeth and Jaws.
II. Further Studies on the Effect of Thiouracil on the
Development of Molar Teeth of Rats.

5. It is felt that one more report should bring this project to a conclusion, and that remaining work on it can be done without spending more money. It is therefore proposed that the grant be suspended at this date.

APPENDIX "N"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: The effect of the endocrines on the teeth and jaws. II. The effect of thiouracil on the bone of the jaws of rats.

Grantee: K.J. Paynter, Dept. of Oral Anatomy, Division of Dental Research, University of Toronto

Project No.: DR 38 Amount of Grant: $1000

This study was designed to determine the effect of hypothyroidism induced by ration of 6-n-propyl-2-thiouracil, on the development of the molar teeth and the jaws of rats. Pregnant female rats were given the drug in the drinking water beginning at about the tenth day of pregnancy. Mothers continued to receive the drug during lactation, and the young were given it in their drinking water after weaning.

It is felt that the investigation has proceeded as far as it can go using the present methods, and that one more report should quite satisfactorily bring it to a close. It is also felt that the rest of the investigation can proceed without spending more money, and therefore I am requesting that this grant be suspended at this date.

As the study has proceeded it has become apparent that the present title is misleading and that the project should more aptly be entitled:

The Effect of the Endocrines on the Teeth and Jaws. II. Further Studies on the Effect of Thiouracil of the Development of the Teeth of Rats.

I hereby request that the project be so renamed.

I have not been able to proceed as far as I had intended with the project during the past six months. I have not had the time to devote to it that I had hoped I might have, and technical difficulties have held up the use of our X-ray equipment. Both of these problems have been solved within the last few weeks.

The results of histological investigations are as follows:

1. There was no detectable difference between control and experimental specimens which were prepared for study within twenty-four hours after birth.

2. Retardation in tooth development did become apparent at later ages. For example, at thirteen days of age the third molar in the experimental animal was still in a bell stage of development.

No organization of odontoblasts or ameloblasts was apparent. The same tooth in a control specimen had started hard tissue formation by this date.

3. In older specimens, (30-36 days) it appeared that eruption may have been more retarded than the other processes which are involved in the formation of teeth. For example, the third molars, in the specimens at thirty-six days had the enamel completely formed, and, with the exception of a small part of it at the cervical third of the tooth, completely matured. The ameloblasts in the cervical region had been reduced in size to that of squamus cells. Yet eruption of the teeth was very much retarded, the external enamel epithelium having not yet met the oral epithelium. Under normal circumstances eruption occurs before maturation of enamel is completed.

Eruption of the first and second molars had occurred but was not as far advanced as it was in the controls.

The periodontal membrane appeared narrower around the roots of the first two molar teeth in the experimental specimens, than it did in the controls. In the bifurcation, particularly of the first molar, the alveolar bone appeared to be almost fused with the cementum deposited on the tooth.

4. It is proposed during the next six months to complete this study, i.e., to complete the X-ray study, to check tooth size, and to compare the state of calcification of the teeth. Results will be presented in a final report to the committee next March.

APPENDIX "O"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: An investigation of the mechanism of repair of the supporting structures of the teeth

Grantee: Dr. Charles H. Best, Banting and Best Department of Medical Research, University of Toronto

Project No.: DR 22 Amount of Grant: $4200

SUMMARY OF WORK

The chief ultimate aim of this work is to develop a treatment to restore the supporting structure of the teeth lost through periodontal disease. In this investigation it has already been demonstrated that soft tissue detached from the teeth can be reattached, and that alveolar process removed can be regenerated. In addition something has been learned of the nature of the regenerative process (Studies I and II). Experimental conditions in these studies have differed in some important respects from the clinical conditions where the loss of supporting structure is brought about by disease.

In Study III, it has been shown that regeneration of supporting structures can be induced where the experimental conditions are quite similar to the clinical. However, the reparative process in this study differed in some respects from that occurring in Studies I and II. A considerable number of cases have been treated to ascertain if the regrowth of the supporting structure in dogs observed in Study III could be induced in humans with periodontal disease. Apparently this seems to be the case. Certainly, a considerable regrowth of supporting structure does occur. The time interval and other features are similar to the regenerative process in the experimental animals. The difficulty of removing from a patient a tooth with a block of bone and surrounding soft tissue, prevents histologic confirmation.

At present it is the writer's opinion that the major method of periodontal treatment to restore lost structure will be based on inducing the type of repair demonstrated in Study III. However, the conditions essential for this slow regrowth (of supporting structure) cannot be attained in certain cases; the rapid type of regrowth occurring in Studies I and II would be more suitable. Studies are now being carried out to investigate the effect on the regenerative process of the various factors of difference between the experimental conditions in Studies I and II and the clinical conditions in periodontal disease. It is premature to report on these studies, but it can be stated that reattachment of the soft tissue wall and regeneration of the alveolar process has been induced in three cases from infected epithelialized pockets in dogs. It is hoped that a report on these studies can be presented for the next meeting of the Committee.

Various minor studies for developing the technique of preparing the various experimental lesions are described in the complete report.

In the March 1953 report, reference was made to the studies in endocrinology being carried on in this department and in the department of Physiology. Hormones have a profound influence upon osteogenesis and thus upon the dental structures. Considerable material of interest to dentistry is available. The jaws of the animals are being collected along with the pertinent data and are sectioned as time permits. We are investigating the possibility of using the dental structures as a biologic indicator in the endocrine studies, which may provide a quantitative evaluation of effects on growth. These studies will be reported at a later date.

APPENDIX "O"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: An investigation of the mechanisms of repair of the supporting structures of the teeth

Grantee: Dr. Charles H. Best, Banting and Best Dept. of Medical Research, University of Toronto

Project No.: DR 22 Amount of Grant: $4200

The chief ultimate aim of this work is to develop a treatment to restore the supporting structures of the teeth lost through periodontal disease. In this investigation it has already been demonstrated that soft tissue detached from the teeth, can be reattached and that alveolar process removed, can be regenerated. In addition something has been learned of the nature of the reparative process (Studies I and II). While considerable information about the supporting structure was learned, these findings were unsuitable for clinical application, as the experimental conditions have differed from those encountered where the loss of supporting structure occurred during the course of periodontal disease.

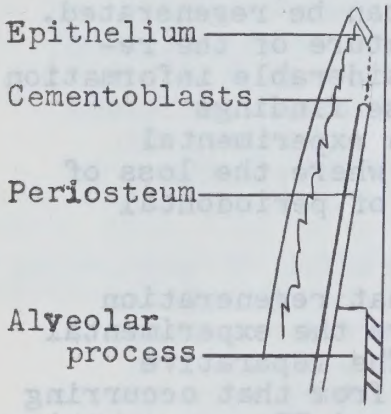
However, in Study III, it has been shown that regeneration of the supporting structures can be induced where the experimental conditions are quite similar to the clinical. The reparative process in this study differed in some respects from that occurring in Studies I and II. For example, in studies I and II, regeneration occurred in approximately 50 days; 18 months was required in Study III. A considerable number of cases have been treated to ascertain if the growth of the supporting structure observed in Study III could be induced in humans with periodontal disease. Apparently this seems to be the case. Certainly a considerable regrowth of supporting structure does occur. The time interval and other features are similar to the regenerative process observed in dogs. However, the difficulty of removing from a patient a tooth with a block of surrounding bone and soft tissue prevents histological confirmation. As an indication of the effect of this experimental work on clinical therapy, in this writer's practice now, the success of treatment is judged more by the amount of regrowth of supporting structure rather than just the control of infection and the establishment of healthy gingival tissues which was the case prior to this investigation. The clinical phase of this investigation will be prepared for publication at a later date.

At present it is the writer's opinion that the major method of periodontal treatment to restore lost supporting structure in practice will be to induce the type of repair demonstrated in Study III. However, the conditions essential for this slow regrowth

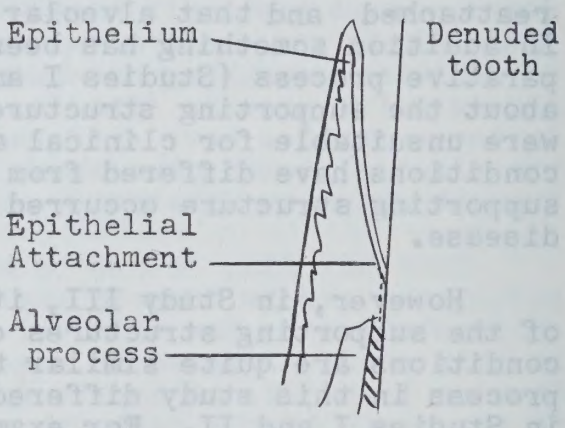
(of supporting structure) cannot be attained in certain individual units; (the tooth and its supporting structure) that it is desirable to retain. The rapid type of repair occurring in Studies I and II would be more suitable. The possibility of reducing or eliminating a periodontal pocket by any method of re-attaching the detached soft tissue wall of the pocket has fascinated the periodontists ever since Younger suggested the idea more than fifty years ago. Many attempts have been made throughout the years to bring about this method of pocket elimination. So far these attempts have met with little success. There is no evidence that a functional regrowth of the supporting structures similar to that occurring in Studies I and II can be induced in periodontal pockets.

There are four main differences between the experimental pocket produced in Studies I and II, and the pathological periodontal pocket. These differences are illustrated in Sketch 1A and 1B.

Experimental Pocket 1A.



Pathological Pocket 1B.



Firstly, in the experimental pocket, the periosteum from the alveolar process removed is still present on the detached gingival flap. Periosteum is not present on the soft tissue wall of the pathological pocket. Secondly, in the pathological pocket the epithelial attachment has migrated rootwise and the inner wall of the detached gingiva is covered with epithelium. The detached gingiva in the experimental pocket is not epithelialized. Thirdly, the pathological pocket becomes infected resulting in a chronic inflammation of the surrounding gingival tissues and fourthly, the denuded cementum has been exposed to the oral fluids for a long time. Experiments are being carried out to investigate these factors as they might effect the regenerative process.

The formation of new cementum necessary for the attachment of the soft tissues to the tooth and the regeneration of the bone are osteogenic processes. It is generally agreed that periosteum is important in osteogenesis. Two studies are being carried out to investigate the part played by the detached periosteum in the regrowth induced in Studies I and II. A third study to investigate the above factor, and the effect of the epithelial lining of the pathological pocket is also being carried on. It is premature to report on these studies, but it is hoped that the work will be far

enough advanced for a report to the next meeting of the Committee. However, it can be now stated that the type of rapid regrowth occurring in Studies I and II, 43 days; has been induced in three cases from epithelialized infected pockets in dogs.

In the previous report on this project, the desirability was discussed of developing a method for preparing periodontal pockets where the area of root exposed by surgical means could be more definitely ascertained in the histologic section. The method used in previous studies was to fill the space created by the removal of a section of alveolar process with periodontal cement pack. This method has many advantages but had the disadvantage that the pack is often dislodged in a week or 10 days. The following methods were tried in a number of cases. Orthodontia banding material was fitted to cover the detached root surface and cemented by means of inlay cement in four cases. These effectively prevented any reattachment, but when examined after a 21-day interval, it was found that the banding material was loose in the pocket. The gingival tissues were markedly inflammed and when a flap was made it was evident that further detachment had occurred with considerable bone resorption. This method of preparing periodontal pockets may be suitable in a later investigation where the effect of infection on the regenerative process (the third factor mentioned above) is being investigated but in the present investigation it proved to be just an additional complicating factor. A number of cases were also prepared, using inlay cement alone to cover the denuded root surface. However, much of the cement became detached and acted as a foreign body in the pocket. A combination of inlay cement and periodontal pack was also used but it was decided that for the present investigation the use of periodontal pack alone to prevent reattachment is the most suitable method. In all, 79 operations on dogs requiring a general anaesthetic were completed to date. Some of the animals have been sacrificed and blocks removed for sectioning.

In the March 1953 report, reference was made to the studies in endocrinology being carried on in this department and in the department of Physiology. Hormones have a profound influence upon osteogenesis and thus upon the dental structures. Considerable material of interest to dentistry is available. The jaws of the animals are being collected along with the necessary data and are sectioned as time permits. Also, we are investigating the possibility of using the dental structures as a biologic indicator in the endocrine studies which may provide a quantitative evaluation of effects on growth. These studies will be reported at a later date.

APPENDIX "P"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Policy of the Associate Committee on Dental Research regarding Publications and Reports

(As adopted by the Committee)
Min.37:10th Meeting, 18/10/49

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1. Grantees in their periodic reports shall inform the Committee of the portions of their work which are to be submitted to some Journal or publisher for publication.

2. Acknowledgement of assistance by the Associate Committee on Dental Research shall appear on the publication. Whenever consistent with the policy of the journal or publication the acknowledgement shall take the form of a footnote saying: "Assisted by a grant from the Associate Committee on Dental Research of the National Research Council of Canada".

3. When such publications appear the grantee shall inform the Secretary of the Associate Committee on Dental Research giving the author or authors, title and reference.

4. The Secretary of the Associate Committee on Dental Research when notified of a publication on work assisted by the Associate Committee on Dental Research shall assign a number to the publication and shall list this number together with the author, title and reference as an appendix to the minutes of the meeting of the Associate Committee on Dental Research.

5. The grantee shall supply, when they become available, twenty-five reprints of each publication for the use of and at the expense of the National Research Council.

6. Reprints of each publication shall be available to each member of the Associate Committee on Dental Research.

APPENDIX "P"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Policy of the Associate Committee on Dental Research
regarding Publications and Reports

(As adopted by the Committee)
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PAPERS PUBLISHED

Committee Paper No.	Title and Author	Reference
1	New aspects of periodontal research, by H.K. BOX	Presented at National Convention of Canadian Dentists, Toronto, 29 May, 1946. (App. "C", 5th Mtg. A.C.D.R., 25 Feb., 1947).
2	Concerning the pathogenesis of periodontal disease, by H.K. BOX	J. Periodontology, 19: 11-20. 1948. (App. "G", 7th Mtg. A.C.D.R., 2 Mar., 1948).
3	The effect of various inorganic salts on the solubility of calcium phosphate, tooth enamel and whole teeth in lactic acid, by J.J. RAE and C.T. CLEGG	J. Dent. Res. 27: 52-53. 1948.
4	Changes in the calcium and phosphate concentrations of saliva and inorganic salt solutions on shaking with calcium phosphate, by J.J. RAE and C.T. CLEGG	J. Dent. Res. 27: 54-57. 1948.
5	An improved method for processing acrylic dentures, by D.R. CHRISTIE	J. Can. Dent. Assoc., 14: 303-317. 1948. (App. "R", 8th Mtg., A.C.D.R., 26 Oct., 1948).
6	The therapeutic properties of periodontal cement packs, by W.J. LINGHORNE and D.C. O'CONNELL	J. Can. Dent. Assoc., 15: 199-205. 1949.
7	Phosphatase in saliva, by J.T. DENTAY and J.J. RAE	J. Dent. Res., Feb. 1949. 4 pp.
8	The treatment of acute oral Vincent's infection, by W.J. LINGHORNE and D.F. STEDMAN	J. Can. Dent. Assoc., July, 1949. 6 pp.
9	A comparison of nine local anaesthetics, by H.S. HAMILTON, B.A. WESTFALL and J.K.W. FERGUSON	J. Pharmacol. Expt. Therap. 94: 299-307 1948.
10	New methods of repairing acrylic dentures without distortion, by D.R. CHRISTIE	J. Can. Dent. Assoc., 15: 582-590. 1949.

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13	Mineralization of the growing tooth as shown by radiophosphorus autographs (17692), by L.F. BÉLANGER and C.P. LEBLOND	Proc. Soc. Expt. Biol. Med. 73: 390-391. 1950.
14	Radio-autographic visualization of bone formation in the rat, by C.P. LEBLOND, G.W. WILKINSON, L.F. BÉLANGER and J. ROBICHON	Am. J. Anat. 86: 2. 1950.
15	Fluoride studies related to the human diet, by MARY P. HAM and M. DOREEN SMITH	Can. J. Research, F. 28: 227-233. 1950.
16	Studies in the regeneration and reattachment of supporting structures of the teeth. I. Soft tissue reattachment, by W.J. LINGHORNE and D.C. O'CONNELL	J. Dent. Res. 29: 419-428. 1950.
17	An examination of the fluoride content of teeth from some Ontario districts, by SHIRLEY R. HARNESS and M. DOREEN SMITH	J. Can. Dent. Assoc., Jan., 1951.
18	Uptake of radioactive calcium by the dental tissues of the young rat, by A. D'IORIO and J.P. LUSSIER	Rev. Canadienne de Biol. 10: 175-180. 1951.
19	Relining acrylic dentures without distortion, by D.R. CHRISTIE	J. Can. Dent. Assoc., July, 1951.
20	Acrylic fillings - General comments and some experimental data, by D.R. CHRISTIE	J. Can. Dent. Assoc., 17: 427-435. 1951.
21	Ammonia production in saliva, by R.M. BALLANTYNE, C.T. CLEGG, J.J. RAE, and F.H. LAWFORD	J. Dent. Res. 30: 385-387. 1951.

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24	Determination of fluoride in water by the aluminum-hematoxylin method, by MARGARET J. PRICE and O.J. WALKER	Anal. Chem., 24: 1593. 1952.

The Secretary of the Committee has a small stock of most of the papers listed and will supply copies to members of the Committee on request.

October, 1953.

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October, 1953.

APPENDIX "Q"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

(i) List of Grantees and Subjects

Grant No.		Grantee	Subject
DR 1	Active	Dr. J.J. Rae, Dept. of Chemistry, University of Toronto	Chemical mechanisms in dental caries
DR 2	Completed	Dr. G.H.W. Lucas, Dept. of Pharmacology, University of Toronto	A study of alveolar oxygen tension and blood oxygen content during nitrous oxide anaesthesia
DR 3	Completed	Dr. H.K. Box, Faculty of Dentistry, University of Toronto	A study of micro-organisms found in deep periodontal pockets and caries lesion as revealed by the electro microscope
DR 4	Completed	Dr. H.K. Box, Faculty of Dentistry, University of Toronto	An investigation of packing material in the treatment of periodontal disease (continuation of investigation previously carried on by Maj. Linghorne)
DR 5	Completed	Major R.L. Twible, Royal Canadian Dental Corps, (later Ontario Research Foundation)	To improve certain properties of the acrylic resin denture base and to effect an economy in denture production
DR 6	Completed	Brig.E.M. Wansbrough, Royal Canadian Dental Corps	To study the effects of altitude acceleration and deceleration on oral tissues
DR 7	Completed	Dr. J.S. Bagnall, Faculty of Dentistry, Dalhousie University	Critical survey of the literature on dental caries
DR 8	Completed	Dr. A.D.A. Mason, Faculty of Dentistry, University of Toronto	A study of the D.M.F. caries rate of the children of Brantford prior to the addition of fluorine to the water supply

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DR 1	Active	Dr. J.J. Rae, Dept. of Chemistry, University of Toronto	Chemical mechanisms in dental caries
DR 2	Completed	Dr. G.H.W. Lucas, Dept. of Pharmacology, University of Toronto	A study of alveolar oxygen tension and blood oxygen content during nitrous oxide anaesthesia
DR 3	Completed	Dr. H.K. Box, Faculty of Dentistry, University of Toronto	A study of micro-organisms found in de periodontal pockets a caries lesion as revealed by the elect microscope
DR 4	Completed	Dr. H.K. Box, Faculty of Dentistry, University of Toronto	An investigation of pac ing material in the treatment of periodon tal disease (continu- ation of investigatio previously carried on by Maj. Linghorne)
DR 5	Completed	Major R.L. Twible, Royal Canadian Dental Corps, (later Ontario Research Foundation)	To improve certain pro- perties of the acrylic resin denture base and to effect an economy denture production
DR 6	Completed	Brig.S.M. Wansbrough, Royal Canadian Dental Corps	To study the effects of altitude acceleration and deceleration on oral tissues
DR 7	Completed	Dr. J.S. Saxmall, Faculty of Dentistry, Dalhousie University	Critical survey of the literature on dental caries
DR 8	Completed	Dr. A.D.A. Mason, Faculty of Dentistry, University of Toronto	A study of the D.M.F. caries rate of the children of Brantford prior to the addition of fluorine to the water supply

Grant No.		Grantee	Subject
DR 9	Completed	Dr. O.J. Walker, Dept. of Chemistry, Dr. H.R. MacLean, Faculty of Dentistry, University of Alberta	An investigation of the effects of certain chemicals on materials in appliances used in prosthetic dentistry
DR 10	Completed	Dr. G.M. Macdonald, Queen Mary Veterans' Hospital, Montreal	Facial prostheses
DR 11	Completed	Dr. R.J. Godfrey, Faculty of Dentistry, University of Toronto	An anatomical, histological and physiological study of the role of the Condyle in Mastication
DR 12	Completed	Dr. A.E.R. Westman, Ontario Research Foundation	Investigation of cements for methacrylate inlays
DR 13	Active	Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Fluoride content of dentine, enamel and alveolar processes of teeth from Sarnia, Brantford and Stratford
DR 14	Completed	Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Fluorine analysis of water and food samples from different areas in Ontario collected and studied in relation to incidence of dental caries
DR 15	Completed	Dr. Norma Ford Walker, Dept. of Zoology, University of Toronto	A comparison of the patterns of eruption of the deciduous teeth in man with rates of growth of the dental arches
DR 16	Completed	Dr. C.H. Best, Dept. of Medical Research, University of Toronto	Studies in vitro of certain fungus-like organisms found in periodontal diseases
DR 17	Completed	Dr. R.F. Shaner, Dept. of Anatomy, University of Alberta	An investigation of the effects of solutions used in operative dentistry on the vital pulps of teeth

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R 11	Completed	Dr. R.J. Godfrey, Faculty of Dentistry, University of Toronto	An anatomical, histological and physiological study of the role of the Condyle in Mastication
R 12	Completed	Dr. A.E.R. Westman, Ontario Research Foundation	Investigation of cements for methacrylate inlays
R 13	Active	Dr. N. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Fluoride content of dentine, enamel and alveolar processes of teeth from Sarnia, Brantford and Stratford
R 14	Completed	Dr. N. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Fluorine analysis of water and food samples from different areas in Ontario collected and studied in relation to incidence of dental caries
R 15	Completed	Dr. Norma Ford Walker, Dept. of Zoology, University of Toronto	A comparison of the patterns of eruption of the deciduous teeth in man with rate of growth of the dental arches
R 16	Completed	Dr. C.H. Best, Dept. of Medical Research, University of Toronto	Studies in vitro of certain fungus-like organisms found in periodontal diseases
R 17	Completed	Dr. K.F. Shaner, Dept. of Anatomy, University of Alberta	An investigation of the effects of solutions used in operative dentistry on the vital pulps of teeth

Grant No.		Grantee	Subject
DR 18	Completed	Dr. H.K. Box, Faculty of Dentistry, University of Toronto	Experimental production of dental caries
DR 19	Completed	Dr. E.A. Sellers, Dept. of Pharmacology, University of Toronto	Tissue toxicity and efficiency of local anaesthetics for topical application and nerve block in dentistry
DR 20	Completed	Dr. Jules Thébaud, Faculty of Dental Surgery, University of Montreal	Improvement of subgingival curettage and surgical techniques used in the treatment of pyorrhea alveolaris
DR 21	Completed	Dr. A.E.R. Westman, Ontario Research Foundation	Investigation of repair techniques for methacrylate dentures
DR 22	Active	Dr. C.H. Best, Dept. of Medical Research, University of Toronto	An investigation of the mechanism of repair of the supporting structure of the teeth and factors affecting the reparative process
DR 23	Active	Dr. C.P. Leblond, Dept. of Anatomy, McGill University	Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radioactive elements
DR 24	Completed	Dr. C.H.M. Williams, Faculty of Dentistry, University of Toronto	The effects of hard and soft diets on the supporting structures of the teeth
DR 25	Completed	Dr. A.E.R. Westman, Ontario Research Foundation	Investigation of methods for the use of methyl methacrylate in direct dental fillings
DR 26	Completed	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	A study of the normal and abnormal physiologic forces of the oral cavity
DR 27	Completed	Dr. J.W. Abbiss, Faculty of Dentistry, Dr. A.G. Nutlay, Faculty of Dentistry, Dalhousie University	A histological study of tooth germ development, both intra and extra alveolar, with a survey of pathological changes in the developing tooth

Grant No.		Grantee	Subject
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rant No.		Grantee	Subject
DR 28	Active	Dr. O.J. Walker, Dept. of Chemistry, University of Alberta	Investigation of methods for destroying the organic matter in teeth bones and other material prior to determination of fluorine content
DR 29	Completed	Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Biological absorption of fluorine
DR 30	Completed	Dr. Norma Ford Walker, Dept. of Zoology, University of Toronto	The study of the closure of space following premature loss of deciduous teeth
DR 31	Completed	Dr. L.B. Jaques, Dept. of Physiology, Mr. G.J. Millar, Dept. of Physiology, University of Saskatchewan	An investigation of nerve block
DR 32	Completed	Dr. A.W. Ham, Dept. of Anatomy, University of Toronto	Investigation of the effect of adrenal cortical hormone imbalances on the various connective tissue structures of the growing incisor teeth of animals
DR 33	Completed	Dr. H.A. Hunter, Faculty of Dentistry, University of Toronto	A study of calcifying potential of calcium oleate on the dental pulp
DR 34	Completed	Dr. A.D'Iorio, Dept. of Physiology, University of Montreal	Studies of calcium metabolism in tooth with the aid of Ca^{45}
DR 35	Active	Dr. J.B. Macdonald, Dept. of Bacteriology, University of Toronto	Bacteriology of periodontal disease I. Experimental fusospirochetal infection with mixtures of pure culture
DR 36	Completed	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	Demineralization of hard tissues as bone and teeth by organic chelating agents

Grant No.		Grantee	Subject
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36	Completed	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	Demineralization of hard tissues as bone and teeth by organic chelating agents

rant No.		Grantee	Subject
DR 37	Active	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	Further studies in the electromyography of the head, face and neck
DR 38	Active	Dr. K.J. Paynter, Faculty of Dentistry, University of Toronto	The effects of the endocrines on the teeth and jaws. II. Further studies on the effect of thiouracil on the development of molar teeth of rats
DR 39	Completed	Dr. A.G. Racey, Faculty of Dentistry, McGill University	Sectioning of teeth and surrounding structures and the effects of potassium deficiency and of pyriodoxine deficiency on the teeth and surrounding structures of dogs
DR 40	Completed	Dr. L.A. Funt, Faculty of Dentistry, University of Toronto	Effect of tooth eruption and function on the growing of the mandible and facial complex on cats
DR 41	Completed	Dr. Harvey Jenkins, Faculty of Dentistry, University of Toronto	A study of the dental arch form and certain of its anatomical relations in a series of clinically excellent dentitions (After Downs (1))
DR 42	Completed	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	Studies in the chemistry of the saliva. II. The reducing property of saliva towards some oxidation-reduction indicators
DR 43	Active	Dr. S.G. Davis, Dept. of Chemistry, Dr. H.R. MacLean, Faculty of Dentistry, University of Alberta	Electro potentials caused by dental alloys
DR 44	Active	Dr. Arthur Harry Craven, Faculty of Dentistry, McGill University	Facial and cranial growth patterns in the rat as revealed by vital staining with alizarine red S

No.		Grantee	Subject
37	Active	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	Further studies in the electromyography of the head, face and neck
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39	Completed	Dr. A.G. Racey, Faculty of Dentistry, McGill University	Sectioning of teeth and surrounding structures and the effects of potassium deficiency and of pyridoxine deficiency on the teeth and surrounding structures of dogs
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42	Completed	Dr. G. Mikiforuk, Faculty of Dentistry, University of Toronto	Studies in the chemistry of the saliva. II. The reducing property of saliva towards some oxidation-reduction indicators
43	Active	Dr. S.G. Davis, Dept. of Chemistry, Dr. H.H. Macleen, Faculty of Dentistry, University of Alberta	Electro potentials caused by dental alloys
44	Active	Dr. Arthun Harry Craven, Faculty of Dentistry, McGill University	Facial and cranial growth patterns in the rat as revealed by vital staining with alizarine red S

ant No.		Grantee	Subject
DR 45	Completed	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	Study of the incidence of malocclusion
DR 46	Active	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	Study of treated cases
DR 47	Active	Dr. H.A. Hunter, Faculty of Dentistry, University of Toronto	The pathological characteristics of experimental fusospirochetal infectio
DR 48	Active	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	The effect of topical application of silicones in protecting teeth against acid solutions, in vitro and dental caries produced in vitro
DR 49	Active	Dr. A.H. Craven, Faculty of Dentistry, University of Toronto	A recording device to determine postural changes of the head in the horizontal plane
DR 50	Active	Dr. J.B. Macdonald, Dept. of Bacteriology, University of Toronto	The cultivation and characteristics of bacteroides nigrescens
DR 51	Active	Dr. R.L. de C.H. Saunders, Prof. of Anatomy, Dalhousie University	Study of human dental pulp blood vessels by X-ray micro-arteriographi methods
DR 52	Active	Dr. L.F. Bélanger, Dept. of Histology and Embryology, University of Ottawa	Mechanism of bone and tooth formation in the light of autoradiography and histochemistry
DR 53	Active	Dr. C.B. Weld, Dept. of Physiology, Dalhousie University	The macrophages in the pulp tissue of the rat incisor and lower jaw

No.		Grantee	Subject
45	Completed	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	Study of the incidence of malocclusion
46	Active	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	Study of treated cases
47	Active	Dr. H.A. Hunter, Faculty of Dentistry, University of Toronto	The pathological characteristics of experimental fusospirochetal infect
48	Active	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	The effect of topical application of silicone in protecting teeth against acid solutions, in vitro and dental caries produced in vitr
49	Active	Dr. A.H. Craven, Faculty of Dentistry, University of Toronto	A recording device to determine postural changes of the head in the horizontal plane
50	Active	Dr. J.B. Macdonald, Dept. of Bacteriology, University of Toronto	The cultivation and characteristics of bacteroides micrococcus
51	Active	Dr. R.L. de C.H. Saunders, Prof. of Anatomy, Dalhousie University	Study of human dental pulp blood vessels by X-ray micro-arteriography methods
52	Active	Dr. L.F. Bélanger, Dept. of Histology and Embryology, University of Ottawa	Mechanism of bone and tooth formation in the light of autoradiograph and histochemistry
53	Active	Dr. C.B. Weld, Dept. of Physiology, Dalhousie University	The macrophages in the pulp tissue of the rat incisor and lower jaw

APPENDIX "Q"7

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

(ii) Summary of Grants by Year 1945-54

Grant	Grantee	1945-46 $	46-47 $	47-48 $	48-49 $	49-50 $	50-51 $	51-52 $	52-53 $	53-54 $
DR 1	Rae	-	900	1000 250	1760 150	1000 550	950	960	1530	1680
DR 2	Lucas	1550	2950	1900	1200 400	325	1550	-	-	-
DR 3	Box	1000	500	500	300	100	-	-	-	-
DR 4	Linghorne R.C.D.C.	-	(Box) 2200 1000	2200	-	-	-	-	-	-
DR 5	Twible R.C.D.C.	-	(Ont.Res. Foundation) 4800	-	-	-	-	-	-	-
DR 6	Coons R.C.D.C.	-	Wansbrough	-	-	-	-	-	-	-
DR 7	Bagnall	200	300	225	-	75	-	-	-	-
DR 8	Ellis (Mason)	-	1000	completed	-	-	-	-	-	-
DR 9	Walker MacLean	-	1000	1000	1100	300	-	-	-	-
DR 10	Macdonald	-	800	completed	-	-	-	-	-	-
DR 11	Godfrey	-	1425 (half-year)	2550	2200	559.07	-	-	-	-
DR 12	Westman	-	-	2550	See DR 21	See DR 25	See DR 25	See DR 25	-	

ASSOCIATE COMPUTER ON DENTAL RESEARCH

(11) Summary of Grants by Year 1945-54

Grants	54-55	53-54	52-53	51-52	50-51	49-50	48-49	47-48	46-47	45-46	Total
Grant 1	-	-	-	-	-	-	-	-	-	-	-
Grant 2	-	-	-	-	-	-	-	-	-	-	-
Grant 3	-	-	-	-	-	-	-	-	-	-	-
Grant 4	-	-	-	-	-	-	-	-	-	-	-
Grant 5	-	-	-	-	-	-	-	-	-	-	-
Grant 6	-	-	-	-	-	-	-	-	-	-	-
Grant 7	-	-	-	-	-	-	-	-	-	-	-
Grant 8	-	-	-	-	-	-	-	-	-	-	-
Grant 9	-	-	-	-	-	-	-	-	-	-	-
Grant 10	-	-	-	-	-	-	-	-	-	-	-
Grant 11	-	-	-	-	-	-	-	-	-	-	-

Grant	Grantee	1945-46 $	46-47 $	47-48 $	48-49 $	49-50 $	50-51 $	51-52 $	52-53 $	53-54 $
DR 13	Smith	-	-	1050	1455	1475	1475	1600	500	-
DR 14	Smith	-	-	1125	1500	-	-	-	-	-
DR 15	Walker N.F.	-	-	3000	3000	2500	800	-	-	-
DR 16	Best	-	-	1100	1500	-	-	-	-	-
DR 17	Shaner	-	-	1000	-	-	-	-	-	-
DR 18	Box	-	-	1350	1620	1620 458.80	-	-	-	-
DR 19	Sellers	-	-	1300	1575	Ferguson- 320		-	-	-
DR 20	Thébaud	-	-	800	750	750	-	-	-	-
DR 21	Westman	-	-	-	5000	-	-	-	-	-
DR 22	Box(later Best)	-	-	-	2200	2400	3800	2300 875 1350	4200	4200
DR 23	Leblond	-	-	-	2200	2200 400	2400	2400	3000	3000
DR 24	Williams	-	-	-	1420	3080	1735	-	-	-
DR 25	Westman (Ont.Res. Foundation)	-	-	-	-	4000	3000	4000	-	-
DR 26	Moyers	-	-	-	-	1300	150	-	-	-
DR 27	Abbiss Nutlay	-	-	-	-	1000	200	-	-	-

Census	Centree	1945-46	46-47	47-48	48-49	49-50	50-51	51-52	52-53	53-54
DR 13	Smith	-	-	1050	1475	1475	1475	1600	500	-
DR 14	Smith	-	-	1125	1500	-	-	-	-	-
DR 15	Walker N.F.	-	-	3000	3000	2500	800	-	-	-
DR 16	Beal	-	-	1100	1500	-	-	-	-	-
DR 17	Shaner	-	-	1000	-	-	-	-	-	-
DR 18	Box	-	-	1350	1650	1650	-	-	-	-
DR 19	Sellers	-	-	1300	1575	Pergus-son 350	-	-	-	-
DR 20	Trebund	-	-	800	750	750	-	-	-	-
DR 21	Weatman	-	-	-	500	-	-	-	-	-
DR 22	Box(Jarret Beed)	-	-	-	2400	2400	3800	2500	1200	4000
DR 23	Ledford	-	-	-	2200	2200	2400	2400	3000	3000
DR 24	Williams	-	-	-	1420	3080	1275	-	-	-
DR 25	Weatman (Ont.Bee's Foundation)	-	-	-	-	4000	3000	4000	-	-
DR 26	Moyers	-	-	-	-	-	150	-	-	-
DR 27	Abbins	-	-	-	-	1300	150	-	-	-
DR ND	Mutay	-	-	-	-	1000	200	-	-	-

Grant	Grantee	1945-46 $	46-47 $	47-48 $	48-49 $	49-50 $	50-51 $	51-52 $	52-53 $	53-54 $
DR 28	Walker O.J. MacLean	-	-	-	-	1000	1000	1000	600	1000
DR 29	Smith	-	-	-	-	1500	375	-	-	-
DR 30	Walker N.F.	-	-	-	-	1200 600	1400 1200	-	-	-
DR 31	Jaques Millar	-	-	-	-	-	1870	188	-	-
DR 32	Ham	-	-	-	-	-	900	-	-	-
DR 33	Hunter	-	-	-	-	-	1500	1600	-	-
DR 34	D'Iorio	-	-	-	-	-	2840	1200 (Lussier)	-	-
DR 35	Macdonald	-	-	-	-	-	2000	2800	3300	3610
DR 36	Nikiforuk	-	-	-	-	-	-	2400 350	1600	-
DR 37	Moyers	-	-	-	-	-	-	960	800	300
DR 38	Paynter	-	-	-	-	-	-	1850	2610	1000
DR 39	Racey	-	-	-	-	-	-	1600	1600	-
DR 40	Funt Sayers	-	-	-	-	-	-	325	-	-
DR 41	Jenkins	-	-	-	-	-	-	300	-	-
DR 42	Nikiforuk	-	-	-	-	-	-	-	1500	-

Grant	Grantee	1945-46 $	46-47 $	47-48 $	48-49 $	49-50 $	50-51 $	51-52 $	52-53 $	53-54 $
DR 43	Davis MacLean	-	-	-	-	-	-	-	150	1025
DR 44	Craven	-	-	-	-	-	-	-	600	1350
DR 45	Moyers	-	-	-	-	-	-	-	1700	-
DR 46	Moyers	-	-	-	-	-	-	-	520	520
DR 47	Hunter	-	-	-	-	-	-	-	1200	2200
DR 48	Nikiforuk	-	-	-	-	-	-	-	-	2240
DR 49	Craven	-	-	-	-	-	-	-	-	400
DR 50	Macdonald	-	-	-	-	-	-	-	-	400
DR 51	Saunders	-	-	-	-	-	-	-	-	1200
DR 52	Bélanger	-	-	-	-	-	-	-	-	1340
DR 53	Weld	-	-	-	-	-	-	-	-	2985

Overall	Competitor	1PM's-4PM	4PM's-4P	4P-4P	4P-5P	5P-5P	5P-5P	5P-6P
DR 43	Davis Malcolm	-	-	-	-	-	150	1025
DR 44	Craven	-	-	-	-	-	600	1350
DR 45	Moyers	-	-	-	-	-	1700	-
DR 46	Moyers	-	-	-	-	-	550	550
DR 47	Hunter	-	-	-	-	-	1200	2200
DR 48	Milford	-	-	-	-	-	-	2240
DR 49	Craven	-	-	-	-	-	-	400
DR 50	Macdonald	-	-	-	-	-	-	400
DR 51	Saunders	-	-	-	-	-	-	1200
DR 52	Belanger	-	-	-	-	-	-	1340
DR 53	Weld	-	-	-	-	-	-	5892

APPENDIX "R"

Suggested Research Projects

By decision of the Committee (Min. 4(x), 13th Mtg.) projects suggested as research topics are listed hereunder for consideration by Committee members:

	Proposed Subject	Suggested by:	Date
1.	Gold inlays over amalgams Little information is available as to whether gold inlays over amalgams cause disintegration of the cement	Dr. M.H. Garvin	October, 1950
2.	A study of root canals of teeth This is a very practical research subject as the results would be of value to every practitioner	Dr. M.H. Garvin	October, 1950
3.	Social aspects of dental disease	Dr. A.C. Burton	September, 1951
4.	The effect of light upon foodstuffs in relation to dentistry	Dr. M.H. Garvin	March, 1952
5.	To test adhesive qualities of acrylic filling material to tooth structure	Dr. M.H. Garvin	September, 1952
6.	Determination of the effect of continued dosage of fluorine on periodontal tissues To make this study it would be necessary to examine the dental records of persons who have lived continuously in an area where fluorine is present in quantity in the water supply.	Dr. M.H. Garvin	March, 1953

APPENDIX "A"

Suggested Research Projects

By decision of the Committee (Min. 4(x), 13th Mtg.) projects suggested as research topics are listed hereunder for consideration by Committee members:

	Proposed Subject	Suggested by:	Date
1.	Gold inlays over amalgams Little information is available as to whether gold inlays over amalgams cause disintegration of the cement	Dr. M.H. Garvin	October, 1950
2.	A study of root canals of teeth This is a very practical research subject as the results would be of value to every practitioner	Dr. M.H. Garvin	October, 1950
3.	Social aspects of dental diseases	Dr. A.O. Burton	September,1951
4.	The effect of light upon foodstuffs in relation to dentistry	Dr. M.H. Garvin	March, 1952
5.	To test adhesive qualities of acrylic filling material to tooth structure	Dr. M.H. Garvin	September,1952
6.	Determination of the effect of continued dosage of fluorine on periodontal tissues To make this study it would be necessary to examine the dental records of persons who have lived continuously in an area where fluorine is present in quantity in the water supply.	Dr. M.H. Garvin	March, 1953

APPENDIX "S"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: Further studies in the electromyography of the head and neck musculature

Grantee: Robert E. Moyers (Dr. H. Jenkins)

Project No.: DR 37 Amount of Grant: $300

SUMMARY OF WORK

The work in the past year has been divided into three separate studies:

A. "An electromyographic study of temporal muscle function and a cephalometric evaluation of its effect on the cranio-facial skeleton in postnormal occlusions". This was the thesis submitted to the School of Graduate Studies, University of Toronto, by Dr. Rowland D. Haryett for the M.Sc., (Dent.) degree. This study has been completed, but publication will not be sought until after it has been entered in the annual research contest of the American Association of Orthodontists.

Both electromyograms and cephalograms were obtained of children exhibiting postnormal occlusions. Samples at two age levels were studied viz., pre-permanent dentition and after the arrival of the second permanent molars. Analysis of the records seems to substantiate the hypothesis that early aberrations in muscle functioning affect the pattern of skeletal growth. The scheme of parallel growth downwards and forwards reported in normal children by Broadbent and Brodie apparently is not seen in children with mandibular retraction. Dr. Haryett has developed in the light of his findings and others pertinent to this problem an hypothesis concerning the formation of Class II malocclusions of a non-genetic origin.

B. "The physiology of centric relation". An electromyographic study of centric relation was undertaken with particular reference to its origin and maintenance. This study is now complete and the final report will be submitted for the next meeting of the Committee.

C. "An electromyographic analysis of various methods of recording centric relation". (Dr. Charles Moses assisted with this study.) This work was correlated with that on the physiology of centric relation, and attempts were made to evaluate the protective neuromuscular reflexes brought into play when attempting to record centric relation. This report is completed and will be submitted to the Committee before the next meeting.

APPENDIX "S"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: Further studies in the electromyography of the head and neck musculature

Grantee: Robert E. Moyers (Dr. R. Jenkins)

Project No.: DR 37 Amount of Grant: $300

SUMMARY OF WORK

The work in the past year has been divided into three separate studies:

A. "An electromyographic study of temporal muscle function and a cephalometric evaluation of its effect on the cranio-facial skeleton in postnormal occlusions". This was the thesis submitted to the School of Graduate Studies, University of Toronto, by Dr. Howland D. Harvett for the M.Sc., (Dent.) degree. This study has been completed, but publication will not be sought until after it has been entered in the annual research contest of the American Association of Orthodontists.

Both electromyograms and cephalograms were obtained of children exhibiting postnormal occlusions. Samples at two age levels were studied via., pre-permanent dentition and after the arrival of the second permanent molars. Analysis of the records seems to substantiate the hypothesis that early aberrations in muscle functioning affect the pattern of skeletal growth. The scheme of paralled growth downwards and forwards reported in normal children by Broadbent and Brodie apparently is not seen in children with mandibular retraction. Dr. Harvett has developed in the light of his findings and others pertinent to this problem an hypothesis concerning the formation of Class II malocclusions of a non-genetic origin.

B. "The physiology of centric relation". An electromyographic study of centric relation was undertaken with particular reference to its origin and maintenance. This study is now complete and the final report will be submitted for the next meeting of the Committee.

C. "An electromyographic analysis of various methods of recording centric relation". (Dr. Charles Moose assisted with this study.) This work was correlated with that on the physiology of centric relation, and attempts were made to evaluate the protective neuromuscular reflexes brought into play when attempting to record centric relation. This report is completed and will be submitted to the Committee before the next meeting.

APPENDIX "T"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: Study of treated cases. (Classification of records)

Grantee: Dr. H. Jenkins for Dr. R.E. Moyers
Report by John Radey

Project No.: DR 46 Amount of Grant: $520

SUMMARY OF WORK

This research project started on October 1st, 1952, and continued through until October 1st, 1953.

1. Tracings and Downs and Wylie analyses were performed for 500 cases.

2. Stock punch cards were typed with the name and number of radiographs and casts. Cases were classified and data marked on punch cards. These were filed alphabetically. Classifications were made into the following categories: age; sex; race; state of health; allergy; anemia; endocrinopathy; co-operation; angle classification; dentition; habits; skeletal dysplasia; hemiatrophy; gingival condition; tooth size; appliance used; congenital absence of teeth; result of type of treatment; extraction cases and mutilated cases.

3. Records - 1207 were put into new envelopes. They were cleaned, marked and filed numerically.

4. Casts of 986 patients were cleaned and the chipped parts were found and patched. These were put into boxes, labelled and filed numerically.

APPENDIX "T"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1953

Subject: Study of treated cases. (An analysis of treated cases)

Grantee: Dr. H. Jenkins for Dr. R.E. Moyers
Report by Dr. Matthew A. Matthews

Project No.: DR 46 Amount of Grant: $520

SUMMARY OF WORK

1. Forty-five Class II, Division I cases treated with labiolingual, edgewise and twin wire appliances were studied by means of cephalometric roentgenograms taken before and after treatment. The effect of treatment on the position and inclination of the maxillary central incisor was analyzed.

2. An analysis of variance was employed to determine the homogeneity of the material at the start of therapy.

3. The treatment procedures used in the edgewise with extraction group demonstrated the highest decrease in the distance of the apex of the upper left central incisor to A point. This was the only group to show a correlation between the decrease in the distances of the apex and the incisal tip of the upper left central incisor to A point.

4. The treatment procedures used in the edgewise without extraction group showed the least decrease in the distance of the apex of the upper left central incisor to A point.

5. Little "bodily" movement of the upper central incisor could be attributed to the edgewise mechanism from the cases studied.

6. The treatment procedures used in the labiolingual without extraction group revealed the mean decrease in the distances of the apex and the incisal tip of the maxillary left central incisor to A point to be more similar than in any other appliance group. However, no correlation coefficient between the two was shown to exist.

7. The treatment procedures used in the labiolingual without extraction and the twin wire without extraction group established mean angles that achieved the closest approach to the normal FP-1 and 1-T mean angles as found by Riedel.

8. The treatment procedures used in the edgewise extraction and non extraction, and the twin wire with extraction groups showed large changes in FP-1 and 1-T angles. These groups also showed

the greatest decrease in the mean distance of the incisal tip of the maxillary central incisor to A point.

9. All appliance groups, with the exception of the twin wire without extraction group, showed a highly significant correlation between the decrease in the distance of the incisal tip of the maxillary left central incisor to A point and the decrease in the FP-$\underline{1}$ angle.

10. It appears that further study regarding the validity and stability of A point should be undertaken.

APPENDIX "U"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

REPORTS ON PROJECTS AT THE 18th MEETING

OCTOBER 22, 1953

Grant No.	Grantee	Reported by
DR 1	Dr. J.J. Rae	Dr. G. Nikiforuk
DR 13	Dr. M. Doreen Smith	Dr. E. Pagé
DR 22	Dr. C.H. Best	Dr. A.W. Ham
DR 23	Dr. C.P. Leblond	Grantee
DR 28	Dr. O.J. Walker	Dr. C.D. Husband
DR 35	Dr. J.B. Macdonald	Grantee
DR 37	Dr. R.E. Moyers (Dr. H. Jenkins)	Dr. J.B. Macdonald
DR 38	Dr. K.J. Paynter	Dr. A.W. Ham
DR 43	Dr. H.R. MacLean Dr. S.G. Davis	Dr. R.H. McDougall
DR 44	Dr. A.H. Craven	Grantee
DR 46	Dr. R.E. Moyers (Dr. H. Jenkins)	Dr. J.B. Macdonald
DR 47	Dr. H.A. Hunter	Grantee
DR 48	Dr. G. Nikiforuk	Grantee
DR 49	Dr. A.H. Craven	Grantee
DR 50	Dr. J.B. Macdonald	Grantee
DR 51	Dr. R.L. de C.H. Saunders	Dr. J.S. Bagnall
DR 52	Dr. L.F. Bélanger	Dr. C.P. Leblond
DR 53	Dr. C.B. Weld	No report

APPENDIX "D"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

REPORTS ON PROJECTS AT THE 18th MEETING

OCTOBER 22, 1953

Grant No.	Grantee	Reported by
DR 1	Dr. J.J. Rae	Dr. G. Nikiforuk
DR 13	Dr. M. Doreen Smith	Dr. E. Page
DR 22	Dr. C.H. Best	Dr. A.W. Ham
DR 23	Dr. C.P. Leblond	Grantee
DR 26	Dr. O.J. Walker	Dr. C.D. Husband
DR 35	Dr. J.B. Macdonald	Grantee
DR 37	Dr. R.E. Moyers	
	(Dr. H. Jenkins)	Dr. J.B. Macdonald
DR 38	Dr. K.J. Paynter	Dr. A.W. Ham
DR 43	Dr. H.R. Maclean	
	Dr. S.G. Davis	Dr. R.H. McDougall
DR 44	Dr. A.H. Craven	Grantee
DR 46	Dr. R.E. Moyers	
	(Dr. H. Jenkins)	Dr. J.B. Macdonald
DR 47	Dr. H.A. Hunter	Grantee
DR 48	Dr. G. Nikiforuk	Grantee
DR 49	Dr. A.H. Craven	Grantee
DR 50	Dr. J.B. Macdonald	Grantee
DR 51	Dr. R.L. de C.H. Saunders	Dr. J.S. Baggall
DR 52	Dr. L.F. Belanger	Dr. C.P. Leblond
DR 53	Dr. G.B. Weld	No report

Members of the Associate Committee on Dental Research
and its Executive, with their terms of appointment

		Term to Expire
*1.	Dr. R.G. Ellis, Chairman, Dean, Faculty of Dentistry, University of Toronto, Toronto, Ont.	31 March, 1954
2.	Dr. E.W.R. Steacie, ex officio, President, National Research Council, Ottawa, Ont.	---

Representing the Dental Profession

3.	Dr. J.S. Bagnall, Dean, Faculty of Dentistry, Dalhousie University, Halifax, N.S.	31 March, 1955
*4.	Dr. E. Charron, Dean of the Faculty of Dental Surgery, University of Montreal, Montreal, Que.	31 March, 1954
5.	Dr. C.D. Husband, Suite 110, Metro Block, Red Deer, Alta.	31 March, 1956
6.	Dr. R.H. McDougall, 1505 Hampshire Road, Victoria, B.C.	31 March, 1955
7.	Dr. A.C. Racey, Associate Professor of Oral Pathology, McGill University, Montreal, Que.	31 March, 1956

Representing the Royal Canadian Dental Corps

*8.	Brig. E.M. Wansbrough, ex officio, Director General of Dental Services, Royal Canadian Dental Corps, Dept. of National Defence, Ottawa, Ont.	---

Representing the Division of Dental Health
Department of National Health and Welfare

9.	Dr. H.K. Brown, ex officio, Chief, Dental Health Division, Dept. of National Health and Welfare, Ottawa, Ont.	---

Representing the Dental Research, Dept. of Veterans Affairs

10.	Dr. L.A. Kilburn, ex officio, Director of Dental Research, Department of Veterans Affairs, Ottawa, Ont.	---

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Representing the Dental Research, Dept. of Veterans Affairs

10.	Dr. L.A. Kilburn, ex officio, Director of Dental Research, Department of Veterans Affairs, Ottawa, Ont.	---

Representing the Basic and Medical Sciences	Term to Expire
11. Dr. J. Aldous, Head of the Department of Pharmacology, Dalhousie University, Halifax, N.S.	31 March, 1956
12. Dr. J.M. Beveridge, Professor, Department of Biochemistry, Queen's University, Kingston, Ont.	31 March, 1956
13. Dr. A.C. Burton, Head of the Dept. of Biophysics, University of Western Ontario, London, Ont.	31 March, 1954
14. Dr. H. Copp, Professor of Physiology, University of British Columbia, Vancouver, B.C.	31 March, 1956
*15. Dr. A.W. Ham, Professor of Anatomy, Faculty of Medicine, University of Toronto, Toronto, Ont.	31 March, 1955
16. Dr. J.D. Hamilton, Professor of Pathology, University of Toronto, Toronto, Ont.	31 March, 1954
17. Dr. J.B. Macdonald, Assistant Professor of Bacteriology, Faculty of Dentistry, University of Toronto, Toronto, Ont.	31 March, 1954
18. Dr. Edouard Page, Director, Department of Nutrition, Medical School, Laval University, Quebec, Que.	31 March, 1955
x19. Mr. S.J. Cook, Secretary, Public Relations Branch, National Research Council, Ottawa, Ont.	---

x Members of the Executive

Representing the Basic and Medical Sciences	Term to Expire
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* Members of the Executive

INITIAL DISTRIBUTION

(i) To members of the

Associate Committee on Dental Research

1. Dr. R.G. Ellis, Chairman
2. Dr. J. Aldous
3. Dr. J.S. Bagnall
4. Dr. J.M. Beveridge
5. Dr. H.K. Brown
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9. Dr. A.W. Ham
10. Dr. J.D. Hamilton
11. Dr. C.D. Husband
12. Dr. L.A. Kilburn
13. Dr. J.B. Macdonald
14. Dr. E. Pagé
15. Dr. A.C. Racey
16. Dr. J.J. Rae ?
17. Dr. E.W.R. Steacie
18. Brig. E.M. Wansbrough
19. Mr. S.J. Cook, Secretary

(ii) To other persons:-

20.-21. Deans of Dental Schools (who are not members of the Committee)

20. Alberta - Dr. W.S. Hamilton
21. McGill - Dr. D. Prescott Mowry

22. Master Copy (General Secretary)
23. Board Room Copy
24. Library

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UNIVERSITY OF TORONTO
FACULTY OF DENTISTRY
OFFICE OF THE DEAN

RECD. DEC 13 1953

ANSWERED _____
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COPY NO. 1

NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS
TWELFTH MEETING
OF THE
EXECUTIVE
ASSOCIATE COMMITTEE ON
DENTAL RESEARCH

OTTAWA

1 MARCH 1954

NATIONAL RESEARCH COUNCIL

Proceedings

Twelfth Meeting

of the

EXECUTIVE

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in Room 360, Chateau Laurier, 8 p.m., Monday, 1 March, 1954.

1. Attendance

Dr. R.G. Ellis, Chairman
Dr. E. Charron
Dr. A.W. Ham
Brig. E.M. Wansbrough

Mr. S.J. Cook, Secretary

Others present were:

Dr. H.K. Brown
Dr. A.G. Racey
Dr. J.B. Marshall (NRC Awards Officer)
Dr. J.B. Macdonald

2. Fellowship application form (Min. 26, 18th Mtg.)

THE CHAIRMAN recalled that the Fellowship Application Form had been discussed at a previous meeting and it had been agreed that the Executive should review this form and report at the March meeting.

DR. HAM said he had given some consideration to this matter as he had been concerned in the revision of certain other fellowship application forms. He thought provision should be made on the form to determine in advance what the applicant proposes to do. He quoted extensively from a letter written by Dr. Ettinger regarding the practise followed in the Division of Medical Research. He suggested that items 19, 20, 21 might be expanded to secure this information.

THE CHAIRMAN thought that the fellowship awards were primarily to encourage and assist training in research and it did not necessarily follow that worthwhile results would be secured in an initial year. When a man was given a grant-in-aid it was expected that he would follow a well-defined line of research and produce results.

After full consideration had been given to this subject it was decided that no change should be made in the fellowship application forms at present, but that the subject should be kept in mind for future consideration.

3. Special Travel Grant Applications (Min. 30, 18th Mtg.)

THE CHAIRMAN said that as a result of the circular notice to grantees stating that provision had been made for the award of a limited number of special travel grants in the amount of $150 each to assist grantees in attending international dental meetings, eight applications had been received. Six of these were for the International Association for Dental Research, one for the American Association of Anatomists at Galveston, Texas, and one for the Histochemical Society at Atlantic City.

Although the Committee had offered seven grants, he thought careful consideration should be given in the selection of those to whom awards were made. He recalled that a letter had been received from Mr. E.R. Birchard, Vice-President (Administration), suggesting that applications for travel grants should be scrutinized very carefully and that the Committee should be prepared to support its recommendations.

Each individual application was then reviewed.

It was agreed to recommend special travel grants of $150 for each of the following:

Dr. L.F. Bélanger (Histochemical Society, Atlantic City)

Dr. J.B. Macdonald (International Association for Dental Research, French Lick, Indiana)

Dr. J.J. Rae (International Association for Dental Research, French Lick, Indiana)

Dr. G. Nikiforuk (International Association for Dental Research, French Lick, Indiana)

The other applicants to whom awards were not made this year were: Dr. K.J. Paynter, Dr. H.A. Hunter, and Dr. H. Jenkins, all of whom wished to go to the International Association for Dental Research; and Dr. B.N. Kropp who would have liked to go to the American Association of Anatomists meeting in Texas.

THE CHAIRMAN suggested and it was AGREED that a tabulation should be included in the proceedings of the Committee showing the names of those who are given special travel grants by the Committee each year.

4. Applications for Grants

The Executive reviewed all applications for grants including renewals and new applications and made the following recommendations.

(i) Renewals approved

Grant No.	Grantee	Amount $
DR 1	Dr. J.J. Rae	1080
DR 13	Dr. M. Doreen Smith	1625
DR 22	Dr. C.H. Best	4200
DR 23	Dr. C.P. Leblond	3500
DR 35	Dr. J.B. Macdonald	2300
DR 37	Dr. H. Jenkins	225
DR 43	Drs. S.G. Davis and H.R. MacLean	250
DR 50	Dr. J.B. Macdonald	400
DR 51	Dr. R.L. deC.H. Saunders	1870
DR 52	Dr. L.F. Bélanger	3470
DR 54	Dr. B.N. Kropp	650
	TOTAL	$19,570

In regard to Dr. Bélanger's application, it may be noted that he asked for $5587.75. This amount included an item of nearly $2000 for the purchase of a cryo-desiccator. The Committee thought that Dr. Bélanger might be given a capital grant of $2000 from 1953-54 funds if he could buy this equipment before the end of the fiscal year. The main sum in the application was reduced accordingly to $3470.

(ii) Renewal deferred

Grant No.	Grantee	Amount
DR 47	Dr. H.A. Hunter	$2620

THE EXECUTIVE was of the opinion that more information was needed on this project before they would be warranted in making a recommendation for a grant.

(iii) New Applications recommended

Dr. M.R. Culbert	$175
Dr. D.J.E. Mitchell	400
Dr. G. Nikiforuk	3200
TOTAL	$3775

(i) Renewals approved

Grant No.	Grantee	Amount
		$
DR 1	Dr. J.J. Rae	1080
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(iii) New Applications recommended

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	Dr. D.J.E. Mitchell	400
	Dr. G. Mikiforuk	3200
	TOTAL	$3775

Both Dr. Culbert and Dr. Mitchell proposed to visit certain institutions for the collection of material and as a consequence most of the funds requested in each case are for travel and subsistence.

It was AGREED to make an exception in the case of these two applicants and to recommend the grants in full instead of paying their travel expenses out of the Committee's travel appropriation. The reason for this was that in each case a small sum had been asked for to cover the purchase of photographic material.

5. Membership of the Committee

THE CHAIRMAN pointed out that there would be five vacancies on the Committee at the end of the present fiscal year.

It was AGREED to recommend the renewal for three years each to 31 March, 1957, of the memberships of Dr. A.C. Burton and Dr. J.B. Macdonald, each of whom has served a single term.

THE CHAIRMAN stated that Dr. Bagnall, who had recently suffered a slight heart attack, had suggested that he might be replaced on the Committee by a younger man. Dr. Bagnall's present appointment expires 31 March, 1955.

It was AGREED that this request should be filed and no action taken on it at present in the hope that Dean Bagnall would still be able to complete his term of office.

THE CHAIRMAN said that Dr. J.D. Hamilton, who has now completed one term as a member of the Committee, had asked that he be not reappointed. Dr. Hamilton is now at Toronto, but was at Queen's University when he was first named to the Committee.

Following discussion on replacements it was AGREED to recommend:

Dr. C.H.M. Williams, in place of Dr. Ellis

Dr. Jean-Paul Lussier, in place of Dr. Charron

Dr. W.S. Hartroft, in place of Dr. Hamilton

THE CHAIRMAN said that since he was retiring from the Committee it would be necessary for the National Research Council to name a new chairman of the Committee. He said that President Steacie had asked that the Committee give consideration to this subject and make a recommendation to Council.

After the subject had been fully reviewed, it was MOVED by Dr. Charron and SECONDED by Brig. Wansbrough

That the Executive recommends to the main Committee that the name of Dr. J.B. Macdonald be suggested to the National Research Council as chairman of the Committee in succession to Dean Ellis. CARRIED.

6. Nomination of Executive

THE CHAIRMAN said that in accordance with a decision reached at the Fourth meeting of the Executive, this group was supposed to be appointed annually although this procedure had not always been followed in practice.

When the subject had been considered, it was AGREED to recommend that the Executive for 1954-55 be comprised as follows:

The Chairman of the Committee
The Secretary of the Committee
Dr. A.W. Ham (reappointed)
Brig. E.M. Wansbrough (reappointed)
Dr. A.G. Racey

7. Secretary of the Committee

THE CHAIRMAN stated that Mr. S.J. Cook was retiring from the Council staff and would therefore not be available to act as Secretary of the Committee as he had done for the past nine years. He said that Mr. Cook had consulted with President Steacie and that as a result Dr. J.B. Marshall, Chief of the Awards Office, was being nominated to succeed Mr. Cook.

The meeting adjourned at 11:20 p.m.

Members of the Associate Committee on Dental Research and its Executive, with their terms of appointment

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* 8.	Brig. E.M. Wansbrough, ex officio, Director General of Dental Services, Royal Canadian Dental Corps, Dept. of National Defence, Ottawa, Ont.	--
	Representing the Division of Dental Health Department of National Health and Welfare	
9.	Dr. H.K. Brown, ex officio, Chief, Dental Health Division, Dept. of National Health and Welfare, Ottawa, Ont.	--

		Term to Expire
Representing the Dental Research, Dept. of Veterans Affairs		
10.	Dr. L.A. Kilburn, ex officio, Director of Dental Research, Department of Veterans Affairs, Ottawa, Ont.	--
Representing the Basic and Medical Sciences		
11.	Dr. J. Aldous, Head of the Department of Pharmacology, Dalhousie University, Halifax, N.S.	31 March, 1956
12.	Dr. J.M. Beveridge, Professor, Department of Biochemistry, Queen's University, Kingston, Ont.	31 March, 1956
13.	Dr. A.C. Burton, Head of the Department of Biophysics, University of Western Ontario, London, Ont.	31 March, 1954
14.	Dr. H. Copp, Professor of Physiology, University of British Columbia, Vancouver, B.C.	31 March, 1956
⁂ 15.	Dr. A.W. Ham, Professor of Anatomy, Faculty of Medicine, University of Toronto, Toronto, Ont.	31 March, 1955
16.	Dr. J.D. Hamilton, Professor of Pathology, University of Toronto, Toronto, Ont.	31 March, 1954
17.	Dr. J.B. Macdonald, Assistant Professor of Bacteriology, Faculty of Dentistry, University of Toronto, Toronto, Ont.	31 March, 1954
18.	Dr. Edouard Pagé, Director, Department of Nutrition, Medical School, Laval University, Quebec, Que.	31 March, 1955
⁂ 19.	Mr. S.J. Cook, Secretary, Public Relations Branch, National Research Council, Ottawa, Ont.	--

⁂ Members of the Executive

Term to Expire

Representing the Dental Research, Dept. of Veterans Affairs

10. Dr. L.A. Kilburn, ex officio, --
Director of Dental Research,
Department of Veterans Affairs,
Ottawa, Ont.

Representing the Basic and Medical Sciences

11. Dr. J. Aldous, 31 March, 1956
Head of the Department of Pharmacology,
Dalhousie University,
Halifax, N.S.

12. Dr. J.M. Beveridge, 31 March, 1956
Professor,
Department of Biochemistry,
Queen's University,
Kingston, Ont.

13. Dr. A.C. Burton, 31 March, 1954
Head of the Department of Biophysics,
University of Western Ontario,
London, Ont.

14. Dr. H. Copp, 31 March, 1956
Professor of Physiology,
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Vancouver, B.C.

* 15. Dr. A.W. Ham, 31 March, 1955
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Medical School,
Laval University,
Quebec, Que.

* 19. Mr. S.J. Cook, Secretary, --
Public Relations Branch,
National Research Council,
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INITIAL DISTRIBUTION

(i) To members of the

Associate Committee on Dental Research

1. Dr. R.G. Ellis, Chairman
2. Dr. J. Aldous
3. Dr. J.S. Bagnall
4. Dr. J.M. Beveridge
5. Dr. H.K. Brown
6. Dr. A.C. Burton
7. Dr. E. Charron
8. Dr. H. Copp
9. Dr. A.W. Ham
10. Dr. J.D. Hamilton
11. Dr. C.D. Husband
12. Dr. L.A. Kilburn
13. Dr. J.B. Macdonald
14. Dr. E. Pagé
15. Dr. A.C. Racey
16. Dr. J.J. Rae
17. Dr. E.W.R. Steacie
18. Brig. E.M. Wansbrough
19. Mr. S.J. Cook, Secretary

(ii) to other persons:-

20.-21 Deans of Dental Schools (who are not members of the Committee)

20. Alberta - Dr. W.S. Hamilton
21. McGill - Dr. D. Prescott Mowry

22. Master Copy (General Secretary)
23. Board Room Copy
24. Library

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12. Dr. L.A. Kilburn
13. Dr. J.D. Macdonald
14. Dr. E. Page
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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS

OF THE

NINETEENTH MEETING

OF THE

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

OTTAWA

1 - 2 MARCH 1954

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NATIONAL RESEARCH COUNCIL

Proceedings

of the

Nineteenth Meeting

of the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in the Council Chamber, National Research Building, 9:30 a.m., 1 - 2 March, 1954.

1. Attendance

Dr. R.G. Ellis, Chairman
Dr. J. Aldous
Dr. J.M.R. Beveridge
Dr. H.K. Brown
Dr. E. Charron
Dr. A.W. Ham
Dr. C.D. Husband
Dr. J.D. Hamilton
Dr. L.A. Kilburn
Dr. J.B. Macdonald
Dr. R.H. McDougall
Dr. A.G. Racey
Brig. E.M. Wansbrough

Mr. S.J. Cook, Secretary

By invitation

Dr. H. Jenkins
Dr. W.J. Linghorne
Dr. J.B. Marshall

Apologies for their absences were received from:

Dr. J.S. Bagnall
Dr. A.C. Burton
Dr. H. Copp
Dr. E. Pagé

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Dr. H. Copp
Dr. B. Pagé

2. Chairman's Remarks

THE CHAIRMAN said that he wished to thank the members and the acting chairman for the successful management of the previous meeting of the Committee in Montreal when he was unfortunately prevented from being present because of illness. He had heard good reports of the meeting.

He said that a letter had been received by the Secretary advising that Dr. J.S. Bagnall had suffered a slight heart attack which made it impossible for him to be present at this meeting. He thought the good wishes of the Committee might be sent to Dr. Bagnall.

In accordance with Committee practice, invitations had been extended to several grantees to be present and to report on their projects. He welcomed Dr. H. Jenkins and Dr. W.J. Linghorne and pointed out that Dr. R.L. deC.H. Saunders, who had also been invited, had thought it would be better to delay the presentation of his report in person to the Committee until his work was further advanced.

A meeting of the Executive would be held in the evening.

3. Message to Dr. Bagnall

It was MOVED by Dr. Charron and SECONDED by Dr. Hamilton

That the Associate Committee on Dental Research has learned with deep regret that illness has made it impossible for Dean Bagnall to be present at this meeting;

The members all wish him a speedy and complete recovery from his present indisposition and look forward to having the benefit of his good counsel at their next meeting.

The Committee AGREED that no action should be taken at the present time on Dean Bagnall's suggestion that he might be replaced on Committee membership.

CARRIED.

4. Minutes of the Eighteenth Meeting

It was MOVED by Dr. Charron and SECONDED by Dr. Kilburn

That the Minutes of the Eighteenth Meeting be taken as read and approved.

CARRIED.

5. Business Arising out of the Minutes

THE CHAIRMAN stated that items arising out of the Minutes included (i) action on the applications received for special travel grants and (ii) revision of the fellowship form. Action on both of these items would be taken by the Executive at the evening meeting.

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CARRIED.

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Consideration of Reports from Grantees

At the meeting, the reports from grantees which were to be presented by guests were heard first, and afterwards the other reports from grantees were read, but for convenience of reference in the proceedings, the reports are arranged in numerical order of projects.

6. DR 1 - Dr. J.J. Rae
Reported by Dr. A.G. Racey

"Chemical mechanisms in dental caries"

A summary and the report appear in APPENDIX "O"

7. DR 13 - Dr. M. Doreen Smith
Reported by Dr. J. Aldous

"Fluoride content of dentin, enamel and alveolar processes of teeth from Sarnia, Brantford and Stratford"

A summary and the report appear in APPENDIX "Q"

DR. ALDOUS in presenting this report made use of a summary table showing the principal data which appear in Tables I - V of the report. The Table is as follows:

	Deciduous teeth			Permanent teeth	
Exposure	Under 3 yr.	7.5 yr.		Under 3 yr.	7.5 yr.
Age	5 - 17	under 8	over 8	5 - 17	under 8
Enamel (% F)	0.0082	0.0118	0.0076	0.0084	0.0130
Dentin	0.0126	0.0308	0.0256	0.0167	0.0363

He pointed out that, as noted in the summary, the rate of gain of fluorine per year of exposure in dentin and enamel of the permanent teeth from Brantford, closely approximates that of the rates of gain of fluorine in the teeth from Stratford.

THE CHAIRMAN said that this project seems to have a direct bearing on Dr. Brown's statistical work and he believed that the results presented in this report by Dr. Smith were more impressive than those given in earlier reports.

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6. DR 1 - Dr. A.A. Rae

Reported by Dr. A.C. Bacey

"Chemical mechanisms in dental caries"

A summary and the report appear in APPENDIX "C"

7. DR 13 - Dr. M. Doreen Smith

Reported by Dr. G. Aldous

"Fluoride content of dentin, enamel and alveolar processes of teeth from Barnia, Brantford and Streetford"

A summary and the report appear in APPENDIX "D"

DR. ALDOUS in presenting this report made use of a summary table showing the principal data which appear in Tables I - V of the report. The Table is as follows:

Exposure	Under 3 yr.	7.5 yrs.		Deciduous teeth	Permanent teeth
Age	5 - 17	under 8	over 8	5 - 17	under 8
Enamel (% F)	0.0082	0.0116	0.0076	0.0084	0.0130
Dentin	0.0126	0.0308	0.0256	0.0167	0.0363

He pointed out that, as noted in the summary, the rate of gain of fluorine per year of exposure in dentin and enamel of the permanent teeth from Brantford, closely approximates that of the rates of gain of fluorine in the teeth from Streetford.

THE CHAIRMAN said that this project seems to have a direct bearing on Dr. Brown's statistical work and he believed that the results presented in this report by Dr. Smith were more impressive than those given in earlier reports.

DR. BROWN said that he supplied the teeth which Dr. Smith used in her study. All of them were from permanently resident children in the areas concerned. He was interested in comparing Dr. Smith's results with those obtained in clinical work. He hoped that someone would undertake X-ray diffraction work on this subject.

DR. CHARRON inquired whether this research was of practical value.

DR. BROWN thought it was unless the Committee was satisfied that the rate at which fluorine is taken up from the water supplies in areas where fluorine occurs naturally, is comparable with that found when the water supply is treated with fluorine artificially. He had been unable until this year to obtain extracted teeth (from artificially fluorinated areas) in which enamel had been formed prior to fluoridation of the water supply.

8. DR 22 - Dr. C.H. Best
Reported by Dr. W.J. Linghorne

"An investigation of the mechanism of repair of the supporting structures of the teeth"

A summary of this report appears in APPENDIX "K"

DR. LINGHORNE, in a comprehensive presentation extending over more than an hour, outlined this research project in detail and reported fully on the results which have been secured during the year, placing special emphasis on the present objective, which is to rebuild the supporting structures of the tooth.

He also touched briefly on endocrine studies which are being carried on concurrently as time permits.

DR. CHARRON said that the advances reported by Dr. Linghorne have been astonishing and he was sure that the results of this investigation would be of great benefit. It was encouraging to note the very definite progress that had been made from the early work.

9. DR 23 - Dr. C.P. Leblond
Reported by Dr. L.F. Bélanger

"Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radioactive elements"

A summary and the report appear in APPENDIX "L"

DR. BELANGER gave a very clear presentation of the work which is being done by Dr. Leblond. The 'conclusion' of the report reads as follows:

> Radioactivity was found in a number of sugars in both the dentin and predentin, which can participate in the periodic acid-Schiff reaction. It is suggested that some change occurs in the sugars at the predentino-dentinal junction resulting in the different degrees of colour intensity after histochemical reactions in the predentin and dentin.

THE CHAIRMAN reported that the work done at McGill by Dr. Leblond and at Ottawa by Dr. Bélanger had formed the basis for a paper which the two workers had been invited to give earlier this year at the New York Academy of Sciences. It was recognized, he said, that an invitation to present a paper before the N.Y. Academy was a signal honour and he offered his congratulations to the authors on having been chosen to present their work.

10. DR 28 - Dr. O.J. Walker
Reported by Dr. C.D. Husband

"Investigation of methods for destroying the organic matter in teeth, bones, and other materials prior to determination of fluorine content"

A summary of this report appears in APPENDIX "S"

DR. HUSBAND said that he had called on Dr. Walker and had discussed this project with him. He reminded the Committee that a memorandum on Dr. Walker's previous report had been prepared following the last meeting of the Committee. There had been some question regarding the results given in Dr. Walker's report. It had turned out on investigation that these discrepancies arose from faulty readings obtained from an old galvanometer. Dr. Walker wanted to know if the Committee would allow him to spend up to about $200 of the funds in his grant this year for the purpose of reconditioning his electrical equipment, in order that dependable results might be secured. Dr. Husband hoped that this could be done as he was quite sure that Dr. Walker was competent to do a very good job on the subject of his study.

It was MOVED by Dr. Macdonald and SECONDED by Dr. Charron

That the Committee recommends that Dr. Walker be permitted to spend up to $200 of his present grant in reconditioning his galvanometer and related equipment in order that he may be able to complete his research project satisfactorily. CARRIED.

11. DR 35 - Dr. J.B. Macdonald
Reported by Dr. Macdonald

"Bacteriology of periodontal disease I. Experimental fusospirochetal infection with mixtures of pure cultures"

A summary and the report appear in APPENDIX "C"

DR. ALDOUS suggested that this work might throw some light on studies of antibiotics which are now being used to kill off various organisms. Perhaps it will prove sufficient to kill one organism and its removal may affect the growth of other organisms which are present. Dr. Macdonald added that he had injected 300 animals with various combinations of organisms excluding spirochaetes but that he had found spirochaetes in two of his experimental animals indicating that these organisms had come from the blood stream of the animals.

THE CHAIRMAN drew attention to a book entitled "The motile non-sporulating anaerobic rods of the oral cavity" by Dr. Macdonald which had been published by the University of Toronto Press recently, and which would now be included as one of the papers arising out of Committee grants. The question of printing this paper had been discussed previously (Min. 11, 16th Mtg. and Min. 10, 17th Mtg.). He thought the Committee might be interested in knowing what Dr. Macdonald had done in this connection.

Dr. Macdonald said that the article was too lengthy for publication either as a single paper or serially in a journal. After trying several different sources he had found that the Canadian Dental Association Research Committee was willing to publish it. The printing had been done by the University of Toronto Press and the distribution is being made by the Canadian Dental Association. He thought that if in future any grantees have contributions which are too long to be published as single papers, they might also consider this means of publication.

It was MOVED by Dr. Charron and SECONDED by Dr. McDougall

That the Committee commend Dr. Macdonald on the publication of the monograph noted above and instruct the Secretary to include this book in the list of Committee publications. CARRIED.

12. DR 37 - Dr. H. Jenkins
Reported by Dr. Jenkins

"Further studies in the electromyography of the head, face and neck"

The summary of this report appears in APPENDIX "J"

THE CHAIRMAN thanked Dr. Jenkins for having come to present his report in person at this meeting. He said that the practice of inviting grantees to report on their own projects was very helpful to the members in forming an opinion on the value of the work.

13. DR 38 - Dr. K.J. Paynter
Reported by Dr. H. Jenkins

"The effect of the endocrines on the teeth and jaws. II. Further studies of the effect of thiouracil on the development of the teeth of rats"

A summary and the report appear in APPENDIX "H"

DR. HAM thought that Dr. Paynter had accomplished about as much as could be expected under this project and hoped that Dr. Paynter might find a better problem on which to work.

14. DR 43 - Drs. S.G. Davis and H.R. MacLean
Reported by Dr. C.D. Husband

"Electropotentials caused by dental alloys"

The summary of this report appears in APPENDIX "P"

DR. HUSBAND said that when he visited the grantees recently he found Dr. Davis very enthusiastic about the problem and with his equipment set up and ready for use. Dr. Davis is a physical chemist, a graduate of McGill and apparently quite a competent worker.

DR. CHARRON thought the Committee should encourage these two men to continue this research project despite the fact that so many calls are being made on them for consulting work and other activities arising out of the present industrial boom in the Edmonton area.

15. DR 47 - Dr. H.A. Hunter
Reported by Dr. J.B. Macdonald

"The pathological characteristics of experimental fusospirochetal infections"

A summary and the report appear in APPENDIX "E"

DR. MACDONALD pointed out that this report by Dr. Hunter terminates the first phase of the study of the pathological characteristics of experimental fusospirochetal infections. The results confirm those of Rosebury et al and conflict with those of Graham. Dr. Hunter planned to continue with a study of the effect of chronic infection using Selye's granuloma pouch technique.

DR. HAMILTON said that this project will probably become a complicated pathological, biochemical and bacteriological problem. He suggested that the applicant might confine himself to determining whether chronic infection can or cannot be established in a granuloma pouch by Dr. Macdonald's fusospirochetal exudates.

DR. HAM said that such work with experimental animals was a very complicated matter. Injection of a tumour for example may bring out many latent infections.

16. DR 48 - Dr. G. Nikiforuk

"The effect of topical application of silicones in protecting teeth against acid solutions in vitro"

A summary and the report appear in APPENDIX "B"

THE CHAIRMAN said that he had invited Dr. Copp to present this report, but that unfortunately Dr. Copp had been unable to attend this meeting. Dr. Copp, however, had read the report by Dr. Nikiforuk and had written regarding it as follows:

> I am particularly sorry that I will not be able to review in person the report by Dr. G. Nikiforuk as requested by Dr. Ellis. I found his report very interesting and believe that silicones may have a very real value in providing an impervious barrier to penetration of dental structures. The problem is certainly worthy of investigation and I think that

DR. HUSBAND said that when he visited the grantees recently he found Dr. Davis very enthusiastic about the problem and with his equipment set up and ready for use. Dr. Davis is a physical chemist, a graduate of McGill and apparently quite a competent worker.

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"The effect of topical application of silicones in protecting teeth against acid solutions in vitro"

A summary and the report appear in APPENDIX "F"

THE CHAIRMAN said that he had invited Dr. Copp to present this report, but that unfortunately Dr. Copp had been unable to attend this meeting. Dr. Copp, however, had read the report by Dr. Nikiforuk and had written regarding it as follows:

> I am particularly sorry that I will not be able to review in person the report by Dr. G. Nikiforuk as requested by Dr. Hilts. I found his report very interesting and believe that silicones may have a very real value in providing an impervious barrier to penetration of dental structures. The problem is certainly worthy of investigation and I think that

Dr. Nikiforuk is to be commended for his work. It is evident, however, that protection is not complete and that many of the preparations are quite ineffective. Indeed, the silicone oils in many cases appear to actually increase the solution of tooth mineral. Unfortunately, I am not competent to judge the actual value of these preparations in dental practice as suggested by Dr. Nikiforuk.

DR. MACDONALD said that the present report was a summary of the investigation. There was nothing much new to add regarding this project since the last meeting of the Committee. This was a short-term project covering a testing procedure prior to a new research on which Dr. Nikiforuk is making an application this year.

DR. HAM inquired whether there was any question of patents arising from this work.

DR. MACDONALD said that the whole subject was being reviewed by Canadian Patents and Development Limited in accordance with the decision recorded in Min. 18, 18th Mtg.

THE CHAIRMAN suggested that at the next meeting of the Committee further inquiry be made as to what has been done in the matter of patents on this subject by Canadian Patents and Development Limited.

17. DR 50 - Dr. J.B. Macdonald
Reported by Dr. Macdonald

"The cultivation and characteristics of Bacteroides nigrescens"

The summary of this report appears in APPENDIX "D"

DR. MACDONALD said that this was a short-term project undertaken to fill a gap in the research program, but that the work might prove of considerable importance because of the 'growth factor' on which information was needed.

18. DR 51 - Dr. R.L. deC.H. Saunders
Reported by Dr. Macdonald

"Study of human dental pulp blood vessels by X-ray micro-arteriographic methods"

The summary of this report appears in APPENDIX "N"

DR. MACDONALD discussed this report briefly drawing attention especially to the combination of microscopic, stereo-photographic and micro-radiographic techniques used by the investigator.

DR. ALDOUS said that prior to the meeting he had visited Dr. Saunders and discussed this project with him. He pointed out that the second picture in the report is a composite of five pictures taken from

the left at an angle of 45° and five other pictures taken from the right at an angle of 45°. Viewed stereoscopically, the figure provides a good 3-dimensional view of the vascular tree and its interconnections as well as its relations to the walls of the pulp cavity and root canal. He said that Dr. Saunders wants to obtain more information on the blood vessels in the network illustrated in Fig. 1.

THE CHAIRMAN thought it would be interesting if he could check the circulation in a live anterior tooth.

19. DR 52 - Dr. L.F. Bélanger
Reported by Dr. Bélanger

"Mechanism of bone and tooth formation in the light of histochemistry and autoradiography"

A summary and exhibit appear in APPENDIX "A"

DR. BELANGER traced the development of this project to date and illustrated his talk by means of a number of excellent colour slides. Since this work is being supported during the present year by grants from both the Dental Committee and the Advisory Committee on Medical Research, he had prepared one report which could be presented to both organizations. (Included as Exhibit I in Appendix "A").

DR. HAM said that this report presented the results of beautiful work.

THE CHAIRMAN added his appreciation to the contribution which is being made by Dr. Leblond and Dr. Bélanger through the application of radioactive materials, in determining the characteristics of a variety of structures.

20. DR 53 - Dr. C.B. Weld

"Macrophages of the incisor and lower jaw"

THE CHAIRMAN said that Dr. Weld's original application was made in April, 1953, and that members of the Committee had been anxious to see Dr. Weld and Dr. Nutlay supported by a grant, but there had been some question as to whether the project proposed justified the amount of the grant requested. There had been an exchange of correspondence as a result of which Dr. Nutlay had submitted a documented account of the literature and this had been brought to the attention of the Executive. It had been decided in September to make a grant of $2985 for seven months to cover the period 1 September 1953, to 31 March, 1954. This was to provide for full payment for the animals and supplies needed and 7/12 of the amount asked for to cover salaries. No report had been presented at the October 1953 meeting of the Committee as the grant had only been made in mid-September.

He said that Dr. Weld had written under date of 29 January, 1954, to the Secretary stating that "no work has been done, no funds have been spent, and no application is being made for a continuation of the grant." He went on to say that Dr. Nutlay,

who was the key man in the project, had made other commitments and was now no longer available.

21. DR 54 - Dr. B.N. Kropp
Reported by Dr. A.W. Ham

"Development of a rapid method for grinding thin sections of plastic-embedded teeth"

A summary and the report appear in APPENDIX "M"

DR. HAM noted that the method described seemed to be of considerable value.

22. Travel Grant to Dr. E.E. Johns (Min. 5(vii), 18th Mtg.)

THE CHAIRMAN said that in accordance with the authority granted him at the previous meeting he had approved a further travel grant to Dr. E.E. Johns to permit him to visit Harvard University and inspect the Hooton Collection of the Peabody Museum. Dr. Johns had submitted a report on this visit which appears in APPENDIX "I"

DR. JENKINS said that interest in this subject had been stimulated by Dr. Moyers' visit to Greece where he had been able to study the teeth of many children living under limited conditions of nutrition. He had been interested in extending this study to an investigation of more primitive man living under similar conditions. The study now embraced these two sections: (i) modern man under primitive conditions and (ii) modern man under modern conditions. It was necessary to obtain large samples in order that reliable results might be secured in the study of skulls. He noted that an application is being made to the Committee this year by Dr. M.R. Culbert for work on a similar project. He understood it was the intention of the investigators to collect the results of these studies and to publish them in a brochure.

THE CHAIRMAN said that while he had been given authority as noted above to make further travel grants to Dr. Johns for travel projects of a similar nature at his discretion, he would like to have the advice of the Committee as to whether this practice should be continued.

It was MOVED by Dr. Charron and SECONDED by Dr. Aldous

That the Committee recommends that the Chairman be authorized to make further grants to Dr. Johns for travel projects similar to those on which he reported at the Seventeenth and Eighteenth Meetings of the Committee. CARRIED.

23. Finances (Min. 28, 18th Mtg.)

(i) Financial standing of Committee 1953-54

THE SECRETARY reported on the financial standing of the Dental Committee as of 31 January, 1954, as follows:

Allotment	$40,700	Grants awarded by Committee	$33,542
Authorized Carry-over from 1953-54 for Commitments	462.18	Travel	1,496.61
		Contingencies	283.58
		Unallotted	5,839.99
	$41,162.18		$41,162.18

THE SECRETARY reported that under new regulations there will be no carry-over of unexpended funds from the fiscal year 1953-54 to the credits for 1954-55. The discontinuance of carry-overs greatly reduced the bookkeeping problem.

NOTE: It is not possible to give figures on amount advanced to each grantee as advances are now made in toto to the university. Approximately 85% of the total of all grants have now been sent to universities for allotment to grantees up to amount authorized by Council.

(ii) Estimates for 1954-55 (Min. 22(iii) 17th Mtg.)

THE CHAIRMAN stated that the estimates for 1954-55 had already been submitted to the National Research Council. He pointed out that the National Research Council is required early each Fall to submit its estimates to the Government for the next fiscal year. Since the Associate Committee would not be holding its autumn meeting in time to consider estimates for 1955-56, he thought this subject should be discussed now. He observed that the figures submitted to Council for the last two years had been in the same total amount, and that each year the funds provided had been adequate to meet the Committee's requirements. He suggested that it might be well to continue this practice in the coming year even though the Committee's expenditures might be slightly increased because of fellowship awards. He proposed that the amount provided for grants be reduced from $32,500 to $30,000 and that allotment for fellowships be increased from $5,000 to $7,500 and that the other items remain unchanged, leaving the total again at $40,700.

DR. HAM suggested that the current surplus might be used in part to purchase equipment provided for in the applications for the coming year.

DR. KILBURN agreed.

DR. CHARRON thought that the Committee had established an excellent reputation for economy and good judgment in the allocation of funds and he considered that a small surplus remaining at the end of the year was to the Committee's credit.

DR. MACDONALD raised the question as to whether adequate provision is being made in grantees' applications for the payment of technicians. It was frequently found that research technicians could not be secured in the salary ranges specified by applicants for grants.

DR. J.B. MARSHALL, Chief Awards Officer, who was consulted, said that the rates paid for research technicians employed under Committee grants were required to conform to the limits imposed by the universities in which the technicians were being employed. He suggested that the employment of a full-time technician by the university at an adequate salary might be preferable to the employment of several part-time technicians by different Committee grantees. The National Research Council had no objection to the pooling of the cost of technicians in this way, but the Council did object to the practice of supplementing a university employee's salary out of Committee appropriations. He thought a good technician was as much entitled to preferred treatment as any other university employee.

It was MOVED by Dr. Husband and SECONDED by Dr. Hamilton

That the Associate Committee on Dental Research respectfully submits the following estimates of its financial requirements for 1955-56 to the National Research Council.

Allotment for	Grants	$30,000
	Fellowships	7,500
	Travel	2,500
	Printing	200
	Contingencies	500
	TOTAL	$40,700

CARRIED.

The meeting adjourned at 5 p.m. to be reconvened at 9:15 a.m., Tuesday, 2 March, 1954.

SECOND SESSION

24. Attendance

Dr. R.G. Ellis, Chairman
Dr. J. Aldous
Dr. E. Charron
Dr. A.W. Ham
Dr. J. Hamilton
Dr. L.A. Kilburn
Dr. J.B. Macdonald
Dr. R.H. McDougall
Dr. A.G. Racey
Brig. E.M. Wansbrough

Mr. S.J. Cook, Secretary

By invitation

Dr. H. Jenkins
Dr. L.F. Bélanger
Dr. J.B. Marshall

The Committee resumed at 9:15 a.m., Tuesday, 2 March, 1954, in the Council Chamber.

SECOND SESSION

24. Attendance

Dr. R.G. Ellis, Chairman
Dr. J. Aldous
Dr. E. Charron
Dr. A.W. Ham
Dr. J. Hamilton
Dr. L.A. Kilbum
Dr. J.B. Macdonald
Dr. E.H. Molyneux
Dr. A.G. Racey
Brig. E.M. Wansbrough

Mr. S.J. Cook, Secretary

By Invitation

Dr. H. Jenkins
Dr. L.F. Belanger
Dr. J.B. Marshall

The Committee resumed at 9:15 a.m., Tuesday, 2 March, 1954, in the Council Chamber.

25. Brigadier E.M. Wansbrough, Q.H.D.S.

THE CHAIRMAN drew attention to the fact that Brig. Wansbrough had received a signal honour from Her Majesty, the Queen, since the last meeting of the Committee and was now entitled to the distinction "Queen's Honorary Dental Surgeon". On behalf of the Committee he congratulated Brig. Wansbrough on having been selected for this particular honour which entitles him to wear the Royal cypher $E_{II}R$ on his shoulder badge. Only four such appointments have been made to officers of the Canadian Forces.

26. Fellowships (Min. 25, 18th Mtg.)

(i) Application from Dr. Clifford Reynolds,
241 Poyntz Ave.,
Willowdale, Ont. $3,000

THE SECRETARY said that Dr. Reynolds is a Canadian, 35 years of age, who had four-and-a-half years' military service with the Canadian Dental Corps during World War II. He is a graduate in dentistry from the University of Toronto (1942) and has completed one-half year as a registered graduate student proceeding to the Master of Science degree in Pharmacology and Biochemistry under Dr. J.K.W. Ferguson.

He proposes to do research on pharmacology as applied to dentistry and has given up private practice to enter research. He is highly recommended by his sponsors, Dr. Roy G. Ellis, Dr. Bruce F. Crocker, and Dr. Charles H.M. Williams.

His application was for only $1800, as he had been given assurance that he would also receive concurrently a studentship from the Canadian Dental Association.

DR. HAMILTON suggested that the amount of the award should be increased to $3,000 on the understanding that he will forego the C.D.A. studentship, or alternatively that he might be employed as an assistant under Dr. J.B. Macdonald's grant.

THE CHAIRMAN drew attention to the NRC regulations governing fellowships in which it is provided under item 7 that successful candidates "are forbidden to hold any position of emolument or to engage in teaching for more than four hours a week" during the tenure of a fellowship.

On further consideration, it was MOVED by Dr. Charron and SECONDED by Dr. Hamilton

That the Committee recommends the award of a dental research fellowship in the amount of $3,000 to Dr. Clifford Reynolds for the year 1954-55 on the understanding that he withdraws his application to the Canadian Dental Association for a studentship to be held concurrently. CARRIED.

25. Brigadier E.M. Wansbrough, O.B.E., D.S.O.

THE CHAIRMAN drew attention to the fact that Brig. Wansbrough had received a signal honour from Her Majesty, the Queen, since the last meeting of the Committee and was now entitled to the distinction "Queen's Honorary Dental Surgeon". On behalf of the Committee he congratulated Brig. Wansbrough on having been selected for this particular honour which entitles him to wear the Royal cypher BEIIR on his shoulder badge. Only four such appointments have been made to officers of the Canadian Forces.

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He proposes to do research on pharmacology as applied to dentistry and has given up private practice to enter research. He is highly recommended by his sponsors, Dr. Roy G. Ellis, Dr. Bruce F. Crocker, and Dr. Charles H.M. Williams.

His application was for only $1800, as he had been given assurance that he would also receive concurrently a studentship from the Canadian Dental Association.

DR. HAMILTON suggested that the amount of the award should be increased to $3,000 on the understanding that he will forego the C.D.A. studentship, or alternatively that he might be employed as an assistant under Dr. G.B. Macdonald's grant.

THE CHAIRMAN drew attention to the NRC regulations governing fellowships in which it is provided under item 7 that successful candidates "are forbidden to hold any position of emolument or to engage in teaching for more than four hours a week" during the tenure of a fellowship.

On further consideration, it was MOVED by Dr. Charron and SECONDED by Dr. Hamilton

That the Committee recommend the award of a dental research fellowship in the amount of $3,000 to Dr. Clifford Reynolds for the year 1954-55 on the understanding that he withdraws his application to the Canadian Dental Association for a studentship to be held concurrently.

CARRIED.

(ii) Application from Dr. Ernest Maarten Madlener,
3 Harbord Street, Apt. 504,
Toronto, Ont. $3,000

THE SECRETARY said that Dr. Madlener was born in Berlin of Dutch nationality in 1919 and has been a resident of Canada since 27 April, 1951. While it is not stated on his application, the Committee was informed that Dr. Madlener served three-and-a-half years with the Netherlands Dental Corps and is, therefore, entitled to the Veterans' preference, in respect of age.

He graduated with honours from the School for the Training of Dentists, Soerabaya, (in what was formerly Netherlands East Indies) in 1940; with honours from Ryksuniversiteit, Utrecht, Holland, 1947; and obtained his D.D.S. with honours from the University of Toronto in 1953, winning the Ed Green Bursary and the James Branston Willmott Scholarship in his second year. He was admitted to the degree of D.D.S. in two years instead of the normal three years demanded from European dentists.

He has carried out some research and published two papers. He is described as an excellent student and is recommended by Dr. J.B. Macdonald (with whom he is working), Dr. G. Nikiforuk, and Dean Roy G. Ellis.

Several members who are acquainted with Dr. Madlener and his work supported the application with enthusiasm.

It was MOVED by Dr. Charron and SECONDED by Dr. Hamilton

That the Committee recommends the award of a dental research fellowship in the amount of $3,000 to Dr. E.M. Madlener for the year 1954-55. CARRIED.

27. Applications for Grants-in-Aid, 1954-55 (Min. 28, 17th Mtg., Min. 27, 18th Mtg.)

THE CHAIRMAN said that the Executive had reviewed all applications for grants including renewals and new applications and had prepared a list of recommendations regarding them for the consideration of the Committee. He pointed out that each member of the Committee should feel quite free to express his views whether they were in agreement with the recommendations put forward by the Executive or not. Each application was then considered by the Committee and action was taken as recorded hereunder.

(i) RENEWALS

Grant No.	Grantee	Amount $
DR 1	Dr. J.J. Rae	1080
DR 13	Dr. M. Doreen Smith	1625
DR 22	Dr. Charles H. Best	4200

(i) RENEWALS (continued)

Grant No.	Grantee	Amount $
DR 23	Dr. C.P. Leblond	3500
DR 35	Dr. J.B. Macdonald	2300
DR 37	Dr. H. Jenkins	225
DR 43	Drs. S.G. Davis and H.R. MacLean	250
DR 50	Dr. J.B. Macdonald	400
DR 51	Dr. R.L. deC.H. Saunders	1870
DR 52	Dr. L.F. Bélanger	3470

Dr. Bélanger's original application was for $5437, but at the meeting he informed the Committee he had received a public health grant in the amount of $9,724.96 from the Department of National Health and Welfare which would enable him to purchase some of the equipment he had asked for in his application to the Committee. He therefore asked that his application be reduced to $3470 for 1954-55.

He would like to ask, however, for an additional $1000 for the purchase of equipment to be used in paper chromatography and paper electrophoresis. This equipment could be purchased in 1953-54 if funds are available.

On discussion it was AGREED to recommend that a capital grant of $1000 be made to Dr. Bélanger from 1953-54 funds.

($1000 from 1953-54 funds)

DR 54	Dr. B.N. Kropp	650
	Total Renewals	$19,570

(4) RENEWALS (continued)

Grant No.	Grantee	Amount
DR 23	Dr. C.P. Leblond	$ 3500
DR 35	Dr. J.B. Macdonald	3300
DR 37	Dr. H. Jenkins	2253
DR 43	Drs. S.G. Davis and H.E. Maclean	750
DR 50	Dr. J.B. Macdonald	400
DR 51	Dr. R.L. deG.H. Saunders	1270
DR 52	Dr. L.F. Bélanger	3470
DR 54	Dr. B.N. Kropp	650
	Total Renewals	$15,570

Dr. Bélanger's original application was for $5437. But at the meeting he informed the Committee he had received a public health grant in the amount of $2,724.00 from the Department of National Health and Welfare which would enable him to purchase some of the equipment he had asked for in his application to the Committee. He therefore asked that his application be reduced to $3470 for 1953-54.

He would like to ask, however, for an additional $1000 for the purchase of equipment to be used in paper chromatography and paper electrophoresis. This equipment could be purchased in 1953-54 if funds are available.

On discussion it was AGREED to recommend that a capital grant of $1000 be made to Dr. Bélanger from 1953-54 funds.

($1000 from 1953-54 funds)

(ii) NEW APPLICATIONS

Grant No.	Grantee	Short Title	Amount $
DR 55	Dr. M.R. Culbert, Assistant Professor of Orthodontics, University of Toronto, Toronto, Ont.	A cephalometric study of maxillary growth as related to statural growth	175

There was some discussion as to whether the $150 in this application, for travel expenses and subsistence during the collection of material, should be awarded as a grant or paid out of the Committee's travel funds.

It was AGREED that since the remainder of the amount asked for is small, the entire amount of $175 should be awarded as a grant-in-aid.

DR 56	Dr. D.J.E. Mitchell, Dept. of Orthodontics, University of Toronto, Toronto, Ont.	Comparative study of palatal height, width and length in ancient and modern crania	400

Here again it was AGREED to make the award in the amount of $400 including $300 for travel as part of the grant.

DR 57	Dr. G. Nikiforuk, Associate Professor of Pedodontics, University of Toronto, Toronto, Ont.	The amino acid composition of enamel protein using paper chromatography	3200

Total of New Applications $3775

(iii) APPLICATION DEFERRED

DR 47	Dr. H.A. Hunter, Associate Professor of Dentistry, University of Toronto, Toronto, Ont.	The pathological characteristics of experimental fusospirochetal infections Part II. Chronic infection	$2620

THE EXECUTIVE had reported that before a recommendation could be made to the Committee regarding this application for a renewal of the grant, it was considered that more information was needed concerning the project.

DR. HAMILTON said that the subject was a very complicated one involving both pathology and chemistry. He thought the applicant would be well advised to limit his research to a study of whether his method would work and how.

DR. MACDONALD agreed with this view.

(ii) NEW APPLICATIONS

Grant No.	Grantee	Short Title	Amount
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There was some discussion as to whether the $150 in this application, for travel expenses and subsistence during the collection of material, should be awarded as a grant or paid out of the Committee's travel funds.

It was AGREED that since the remainder of the amount asked for is small, the entire amount of $175 should be awarded as a grant-in-aid.

DR 56	Dr. D.J.E. Mitchell, Dept. of Orthodontics, University of Toronto, Toronto, Ont.	Comparative study of palatal height, width and length in ancient and modern crania	400

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DR 57	Dr. G. Nikiforuk, Associate Professor of Pedodontics, University of Toronto, Toronto, Ont.	The amino acid composition of enamel protein using paper chromatography	3200

Total of New Applications $3775

(iii) APPLICATION DEFERRED

DR 47	Dr. H.A. Hunter, Associate Professor of Dentistry, University of Toronto, Toronto, Ont.	The pathological characteristics of experimental tuberculous infections Part II. Chronic infection	$262

THE EXECUTIVE had reported that before a recommendation could be made to the Committee regarding this application for a renewal of the grant, it was considered that more information was needed concerning the project.

DR. HAMILTON said that the subject was a very complicated one involving both pathology and chemistry. He thought the applicant would be well advised to limit his research to a study of whether his method would work and how.

DR. MACDONALD agreed with this view.

THE CHAIRMAN said the Committee should have some assurance from the applicant regarding the limitation of his project, and when this information had been obtained, the Executive might take action on the application. He asked the Secretary to write to Dr. Hunter.

DR. HAM suggested that if Dr. Hamilton were willing to give advice to the applicant it would be very helpful.

DR. HAMILTON agreed to do so.

28. Summary of Applications (Min. 29, 17th Mtg.)

	No.	Value
(i) Renewal applications approved	11	
(ii) Total value		$19,570
(iii) New applications approved	3	
(iv) Total value		$3,775
(v) Total awards	14	
(vi) Deferred	1	$2,620
(vii) Capital grant from 1953-54 funds	1	$1,000

It was MOVED by Brig. Wansbrough and SECONDED by Dr. Aldous

That the recommendations regarding renewals of grants and the award of new applications listed above be approved for submission to the National Research Council. CARRIED.

29. Special Travel Awards (Min. 30, 18th Mtg.)

THE CHAIRMAN stated that eight applications had been received for special travel grants whereby grantees might be assisted to attend scientific meetings such as the International Association for Dental Research. The Committee had previously recommended that provision be made for seven such grants limited in each case to $150 and subject to the usual regulations covering travel appropriations.

He said that the Executive had considered this subject at some length and had decided to recommend that only four awards be made in the coming year. In the discussion, reference had been made to a memorandum received last year from the Vice-President (Administration), Mr. E.R. Birchard, suggesting that very careful scrutiny should be given to applications for travel grants.

The Executive had expressed the view that, in selecting candidates for the award of travel grants, a primary requirement should be that the applicant is doing selective reporting of outstanding work on a project supported by the Committee. There was some need to be cautious in making travel grants although the Executive was convinced of their value.

The Executive recommended that travel awards not to exceed $150 each be made in 1954-55 as follows:

(i) to attend the International Association for Dental Research

Dr. J.B. Macdonald

Dr. G. Nikiforuk

Dr. J.J. Rae

(ii) to attend the meeting of the Histochemical Society in Atlantic City, 15-17 April, 1954,

Dr. L.F. Bélanger

It was MOVED by Dr. Aldous and SECONDED by Dr. Kilburn

That the recommendations for four travel grants listed above be approved. CARRIED.

It was AGREED to include as an appendix in the proceedings a tabulation showing the names of the grantees given special travel grants each year.

THE CHAIRMAN reported that he had personally authorized the attendance of Dr. C.P. Leblond at a meeting of the New York Academy of Sciences at which Dr. Leblond and Dr. Bélanger had been invited to give a joint lecture on the autoradiographic work they are doing under grants from the Dental Committee. He considered that the invitation from the New York Academy was a high honour to the two research workers and that their contribution was a tribute to the activities of the Associate Committee on Dental Research.

30. Authorization for Air Travel, 1954-55 (Min. 31, 17th Mtg.)

It was MOVED by Brig. Wansbrough and SECONDED by Dr. Hamilton

That air travel to meetings of the Committee in 1954-55 be authorized for all members of the Committee, to be used at their discretion. CARRIED.

31. Membership of the Committee (Min. 32, 17th Mtg. Min. 5(viii) 18th Mtg.)

THE CHAIRMAN asked the Secretary to report on the status of Committee membership.

THE SECRETARY said that the Chairman, Dr. R.G. Ellis, and Dr. E. Charron had each completed three full terms of three years. He pointed out that the usual custom is not to appoint a member for more than two consecutive terms, but that the third term had been given to Dr. Ellis and Dr. Charron at the special request of former President C.J. Mackenzie, as a mark of recognition for their contribution in the establishment and

growth of the Committee and in order to provide for a continuance of their service during the last three years.

Three other members had each completed one three-year term, and were therefore eligible for reappointment. These were: Dr. A.C. Burton, Dr. J.D. Hamilton, and Dr. J.B. Macdonald.

Dr. J.S. Bagnall who had served one part-term (1950-52) and was now serving his first full term (1952-55) had written to suggest that consideration might be given to replacing him by a younger man at this meeting, but the Executive had recommended that no action be taken in regard to this request at the present time since it was their hope that Dr. Bagnall might soon be quite well again and able to carry on until the end of his term.

Dr. J.D. Hamilton who had been appointed to the Committee while he was a member of the staff of Queen's University and had subsequently moved to Toronto, had requested that he be not reappointed at the end of his present term which expires 31 March, 1954.

The Executive had considered the matter of Committee membership very carefully and had agreed upon the following recommendations.

(i) That Dr. A.C. Burton and Dr. J.B. Macdonald be recommended for a further appointment of three years each to 31 March, 1957.

(ii) That invitations to serve on the Committee for a three-year term to 31 March, 1957, be extended to:

Dr. C.H.M. Williams,
Associate Professor
of Periodontology,
Faculty of Dentistry,
University of Toronto,
Toronto, Ont. (Replacing Dr. Ellis)

Dr. Jean-Paul Lussier,
Assistant Professor,
Faculty of Dental Surgery,
University of Montreal,
Montreal, Que.
(Replacing Dr. Charron)

Dr. W.S. Hartroft,
Banting and Best
Department of Medical Research,
University of Toronto,
Toronto, Ont.
(Replacing Dr. Hamilton)

(iii) That Dr. J.B. Macdonald be nominated as Chairman of the Committee in succession to Dr. R.G. Ellis, resigned.

(iv) That the Executive for 1954-55 be comprised as follows:

The Chairman of the Committee
The Secretary of the Committee
Dr. A.W. Ham (reappointed)
Brig. E.M. Wansbrough (reappointed)
Dr. A.G. Racey

ACTION

It was MOVED by Dr. Hamilton and SECONDED by Dr. McDougall

(a) That the Committee approve the recommendations from the Executive that Dr. Burton and Dr. Macdonald be reappointed for a further term of three years and that no action be taken at present on Dr. Bagnall's request that he be replaced on the Committee. CARRIED.

It was MOVED by Dr. Aldous and SECONDED by Dr. McDougall

(b) That invitations to serve as members of the Committee for a three-year term be extended to Dr. C.H.M. Williams, Dr. Jean-Paul Lussier, and Dr. W.S. Hartroft. CARRIED.

It was MOVED by Dr. McDougall and SECONDED by Dr. Kilburn

(c) That the nomination of Dr. J.B. Macdonald as Chairman of the Committee be recommended to the National Research Council. CARRIED.

It was MOVED by Dr. Hamilton and SECONDED by Dr. Aldous

(d) That the Executive for 1954-55 be comprised of The Chairman, The Secretary, Dr. A.W. Ham, Brig. E.M. Wansbrough, and Dr. A.G. Racey. CARRIED.

32. Suggested Research Projects (Min. 34, 17th Mtg.)

DR. MACDONALD doubted the value of continuing to carry a list of suggested research projects in the proceedings of the Committee.

THE CHAIRMAN said he thought it was unlikely that research workers would find these suggestions helpful. Most of those undertaking research had their own ideas as to projects.

It was MOVED by Dr. Macdonald and SECONDED by Dr. Charron

That publication of the list of suggested research projects in the proceedings of the Committee be discontinued, but that the present list be put on file in the Secretary's office for reference. CARRIED.

33. Dr. Bagnall's Bibliography (Min. 5(ii) 18th Mtg.)

THE SECRETARY reported that the total distribution of this Bibliography had amounted to 261 copies up to March 1954. There remain on hand in the Secretary's office 59 copies as compared with 66 in October 1953.

DR. MACDONALD said there must be a large amount of new information on this subject now and he wondered whether Dr. Bagnall had considered the preparation of a supplement to his large book, or whether anyone else was thinking of it. After some discussion, it was AGREED that further consideration of this suggestion might be deferred and brought to the attention of the incoming Executive to discuss with Dr. Bagnall.

THE SECRETARY said that he had in his office a copy of "A survey of the literature of dental caries" published in 1952 by the National Academy of Sciences - National Research Council, Washington, D.C. It was AGREED that this book should be filed with the Committee papers and mentioned as an available reference in the list of publications of the Committee.

34. Appreciation of Services

It was MOVED by Dr. Hamilton and SECONDED by Brig. Wansbrough

That the Associate Committee on Dental Research has learned with regret of the retirement of Mr. S.J. Cook as Secretary of the Committee, and wishes to record its appreciation of the valuable services he has rendered in aiding the Committee since its inception, in every aspect of its activities, and in contributing very materially to the success of the Committee's functions. The Committee extends to Mr. Cook its best wishes for many years of good health and happiness.

DR. CHARRON speaking to this motion said that he wishes to add his personal tribute to the success that had been achieved in the Committee's work under the guidance of Dr. R.G. Ellis. He recalled that former President Mackenzie and President Steacie had both expressed high approval of the Committee's program of work and the results which had been secured in the promotion of dental research in Canada. The profession owed much to the Chairman and he wished to add that it had always been a great pleasure to work with him.

35. Secretary of the Committee

THE CHAIRMAN announced that owing to his impending retirement from the Council staff, Mr. S.J. Cook would not be available to act as Secretary of the Committee after this meeting. In consultation with President Steacie, on this matter, Mr. Cook had been informed that it was proposed to submit the name of Dr. J.B. Marshall, Chief of the Awards Office, to Council as the new Secretary of the Committee.

THE SECRETARY presented Dr. Marshall to the Committee and expressed the opinion that Dr. Marshall's long experience in Committee work and his present duties as head of the Awards Office, made him particularly suitable as Secretary of the Dental Committee.

THE CHAIRMAN welcomed Dr. Marshall.

36. Date of Next Meeting (Min. 33, 18th Mtg.)

On motion of Dr. Kilburn SECONDED by Dr. Aldous

It was AGREED that the date of the next meeting should be tentatively set for Monday, 25 October, 1954.

The meeting adjourned at 12:30 p.m.

APPENDIX "A"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: Mechanism of bone and tooth formation in the light of histochemistry and autoradiography

Grantee: Dr. Léonard F. Bélanger

Project No.: DR 52 Amount of Grant: $1200

SUMMARY OF WORK

The autoradiographic tracing of Sulphur 35 tagged material in sections treated by routine histological technique have revealed that sulphur is retained mainly as sulfopolysaccharides in a variety of structures. An abundant amount of such has been found in the cartilage, bone, dentine, and enamel and is closely related to the phenomenon of mineralization. The preliminary in vitro exchange seems to indicate that this material is present along with the crystal lattice and seems responsible for decrease in P^{32} in vitro exchange of adult mineralized tissues. The calcium 45 tracing of growth in the dentine of incisors of pigs has revealed a pattern of growth comparable to that of the previously described incisor of rat. Sodium fluoride introduced in the diet for 30 days or more in proportions of 200 and 1000 p.p.m. has produced a marked retardation of growth as gauged by mineral deposition and also an irregular wavy pattern of dentine apposition. (See Exhibit I attached).

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EXHIBIT I

Report to

The Advisory Committee on Medical Research

Division of Medical Research

National Research Council, Canada

SUBJECT: Mechanism of bone and tooth formation in the light of histochemistry and autoradiography.

AUTHOR: Léonard F. Bélanger

LABORATORY: Department of Histology and Embryology, School of Medicine, University of Ottawa.

GRANT NO: MP-174

PURPOSE: To continue investigations of the fundamental patterns of growth in the bones and teeth of Rats and Hamsters, particularly in regard to mineralization.

EXPERIMENTAL PROCEDURE: In the course of the past year young suckling Rats and Hamsters were injected with Calcium 45 as $CaCl_2$ on the one hand. Other series were injected with Sulphur 35 as H_2SO_4. These animals were sacrificed at time intervals ranging from one hour to several weeks thereafter and the tissues fixed in formaldehyde-ethanol were embedded in either celloidin or plastic (Ward's Bio-Plastic) and then cut or ground and prepared for autoradiography by the improved inversion technique, after coating with fluid photographic emulsion (Bélanger, Nature, 1952).

A series of teeth from Pigs, Calves, Sheep injected with Calcium 45 or Sulphur 35 and also some of which were treated with various concentrations of Sodium Fluoride over various periods of time, were provided by Dr. C.L. Comar and his group at the University of Tennessee-Atomic Energy Commission, Agricultural Research Unit, at Oak Ridge Tennessee. This material was treated as above and examined by autoradiography.

Some rat material was incubated in vitro with hyaluronidase and the results were appreciated by autoradiography of this material and controls and also by comparative studies utilizing toluidine blue metachromatic staining.

Some human bones and teeth were also studied autoradiographically after in vitro exchange with various isotopes (P^{32}, Ca^{45} and S^{35}, Bélanger, J. Dent. Res., 1952).

RESULTS: Sulphur 35 Work: The results of the widespread study with S^{35} have given rise to six papers so far, the titles and summaries of which follow. Reprints of papers out of press are also included.

1. Autoradiographic Detection of S^{35} in the Membranes of the Inner Ear of the Rat, Science, 118, 520-521, 1953.

"In conclusion, it seems that all three membranes synthesize organic sulfur compounds. The cupula and the membrana tectoria show a larger concentration of S^{35} than the otolithic membrane. The sulfur compounds formed do not have a rapid turnover. The histochemical tests do not reveal the presence of keratin in the inner ear but point strongly to the existence of sulfomucopolysaccharides in all three types of membranes. In the tectorial and otolithic membranes at least part of these were found to be hyaluronidase resistant."

2. Autoradiographic and Histochemical Studies of the Entry and Incorporation of Sulphur in Growing Bones and Teeth of the Rat, Abstracts and Communications, XIX International Physiological Congress, Montreal, 1953.

"S^{35} as H_2SO_4 separated isotope was introduced subcutaneously into 4 day-old rats which were then sacrificed at intervals of 1,2 and 24 hrs. and on alternate days up to 8 days thereafter. Autoradiographs were prepared by the inversion technique from alcohol-formaldehyde fixed tissues and from sections of same, pretreated with formic-citrate at pH 4.9 or incubated with hyaluronidase. The cartilage showed an intense accumulation of S^{35} in the proliferating cells at 1-2 hrs. The sulphur was then seen in the capsule and cartilage matrix at 24 hrs. and by the end of the second day it was mostly in the areas of hypertrophy and calcification indicating growth movement towards the epiphysial plate. An overall weak, persistent reaction was recorded also over cartilage, tendons, ligaments and aponeuroses. The initial picture over bone was one of general, rather large intake. After 2 days only a small autoradiographic band at the periosteal junction and a weak reaction over the new spicules were apparent. In teeth, there was also an overall intense reaction over dentine, which had disappeared after 2 days, to make place for a weak band which migrated with time towards the dentino-enamel junction. Over enamel, there was a strong initial linear reaction at the ameloblast border, which moved slowly and diffusedly towards the inner regions of enamel with time.

After acid demineralization, the autoradiographic picture persisted over cartilage and areas of growth in bone, dentine and enamel. The overall initial reaction over bone and dentine disappeared with the minerals.

With hyaluronidase incubation, the reaction disappeared rapidly from bone and dentine and slowly from cartilage according to a pattern probably indicative of the original concentration. The picture persisted over enamel after it had disappeared from dentine, bone and cartilage."

3. Autoradiographic Detection of Sulphur-35 Synthesis by the Mucous Neck Cells of the Rat's Stomach, Nature, 172, 1150, 1953.

"These experiments indicate that there is a chemical difference between the secretion of the surface cells and that of the mucous neck cells. These have the ability to synthesize rapidly an organic substance containing sulphur and retained by alcohol-formaldehyde fixation. Boström and Odeblad have recently demonstrated that, following an injection of radiosulphate, the uptake of sulphur-35 is highest in structures containing mucopolysaccharides. It is thus reasonable to conclude that the mucous neck cells produce a mucoprotein containing a sulphomucopolysaccharide, and that in comparable circumstances this substance is not present in the surface cells. The product of the mucous neck cells is totally excreted at the end of two days, and it is found in the chyme where it retains its identity after histological fixation. Work is in progress to establish comparisons between the various portions of the stomach and other regions of the gastro-intestinal tract."

4. Autoradiographic Visualization of S^{35} Incorporation and Turnover by the Mucous Glands of the Gastro-Intestinal Tract and Other Soft Tissues of Rat and Hamster, in press with Anatomical Record.

"Following a subcutaneous injection of S^{35} labelled H_2SO_4, the radiosulfur has been localized by autoradiography to various tissues and organs, with a large range of relative radioactivity.

The mineralizing tissues and the mucous glands have exhibited the largest synthesis, with the exception of the surface cells of the stomach and the Brünner glands of the duodenum which do not seem to pick-up radiosulfur.

The mast cells of connective tissue and all areas rich in collagen have taken in radiosulfur.

The region of the basement membrane in the gastro-intestinal tract and genito-urinary system have also shown evidence of sulfate fixation.

The medulla of the kidney and the megakaryacytes of the spleen have also given positive S^{35} autoradiographs.

All these localizations seem related to the building up and concentration of sulfopolysaccharides and stain metachromatically with toluidine blue.

The radiosulfur has disappeared within 2 days from all mucous glands except from the olfactory mucosa. The connective tissue seems to have lost most of its organic bound sulfate at the end of that period with the exception of the mast cells which were still strongly positive after 4 days."

5. Autoradiographic Detection of Radiosulfate Incorporation By The Growing Enamel of Rats and Hamsters, in press with J. Dent. Res.

"Radiosulfate administered subcutaneously to young rats and hamsters has been detected autoradiographically in the ameloblasts and new enamel during the period of growth. It seems to be synthesized into an acid sulfo-mucoprotein which appears in the matrix before keratin and disappears progressively thereafter.

This substance is acid fast and hyaluronidase resistant.

When present in large amounts it may be an inhibitor of mineralization.

Its formation and secretion is concomitant with alkaline phosphatase activity.

During the maturation period of the enamel, a concentration of S^{35} appears at the dentino-enamel junction and diffuses upward. It is likely coming from the dentine and it might represent an invasion of sulfopolysaccharides favoring mineralization."

6. Autoradiographic Visualization of the Entry and Transit of S^{35} in Cartilage, Bone and Dentine of Young Rats and the Effect of Hyaluronidase In Vitro, submitted for publication in the Canadian Journal of Biochemistry and Physiology.

"The fate of radiosulfate introduced in young suckling rats has been followed by autoradiography of formalin-ethanol fixed celloidin sections. In the cartilaginous epiphyses of long bones, the radioelement has been detected first in the cells, particularly at the hypertrophic level, then in cells and matrix, finally in matrix exclusively. The incorporated radiosulfate was later displaced towards the ossification area by the growth process. During this migration, the relative intensity of the autoradiograph decreased progressively. This series of events was interpreted as visualization of chondroitin sulfate synthesis, secretion and utilization or turnover. The S^{35} labelled substance was rapidly hydrolyzed by hyaluronidase except in areas of tendon insertions. In bone and dentine, an overall initial exchange picture was replaced by a growth line of acid fast, hyaluronidase labile sulfated material which became displaced along with bone salts by further growth. This material was metachromatic with toluidine blue. It is thought to be also chondroitin sulfate and its possible relationship to mineralization has been discussed."

RESULTS: Calcium 45 Work: Rats and Hamsters: No report has yet appeared on these experiments, particularly on account of the fact that in the enamel there seems to be a discrepancy between the results obtained with phosphorus and calcium tracers which would perhaps indicate a presence of calcium in other form than the mineral combination with the phosphate. Experiments to control this and other points are to be continued during the forthcoming year.

RESULTS: Calcium 45,Pigs: Autoradiographic Visualization With Ca^{45} of Normal Growth of The Incisor of Pigs and the Effect of Fluorine Feeding, L.F. Bélanger, W.E. Lotz, W.J. Visek and C.L. Comar, in press with Anatomical Record.

"The entry and transit of a tracer dose of Ca^{45} has been visualized by autoradiography in the incisor teeth of pigs on normal diet and of pigs fed 200 and 1000 p.p.m. sodium fluoride.

In the normal animals, an autoradiographic band has been seen first over the formative areas of dentine and 'moving' outwards with time at a rate inversely proportional to age of the tissue, to eventually 'disappear'.

Over cementum there was a similar band moving inwards.

Pigs fed 200 p.p.m. sodium fluoride and feeding ad lib. did not exhibit any marked differences from the previous pattern.

Pigs fed 200 p.p.m. sodium fluoride and maintained on par feeding, showed retardation of growth of dentine and cementum and an irregular, slightly wavy pattern of the radioactive band over dentine.

All pigs fed 1000 p.p.m. sodium fluoride exhibited marked retardation of growth of dentine, cementum, and alveolar bone and a coarse, wavy pattern of the autoradiographic band over dentine.

These defects are apparently related to toxicity and abnormal mineral deposition.

Geiger counts over the crown and root portions of the teeth exhibited marked differences between these regions in all teeth, but no significant differences between comparable portions of teeth from each group, neither between the 10 and 45 day stages."

In vitro exchange experiments with human and other material:

Autoradiographic Visualization of In Vitro Exchange in Teeth, Bones and Other Tissues, Under Various Conditions, J. Dent. Res. 32, 168-176, 1953.

1. Autoradiograms of in vitro exchanged P^{32}, Ca^{45}, and S^{35} in teeth and bones have been obtained.

2. Exchange behavior of young bones and teeth in vitro is comparable to the early autoradiographic patterns after injections of P^{32} or Ca^{45} in vivo.

3. Young normal tissues exchange at a greater rate than older tissues.

4. Areas of new growth in a bone tumor and around a bone graft exchange more than normal bone.

5. Recent calcified lesions of hyaline cartilage and muscular arteries exchange at a higher rate than older lesions.

6. In adult human teeth, cementum exchanges more than dentine. Dentine exchanges more than enamel.

7. In the enamel of certain adult human teeth, caplike regions proximal to the dentino-enamel junction exchange heavily but show no difference in radiopacity.

8. Following hyaluronidase incubation, there is a progressive increase in exchange over dentine.

9. The process of dentinal caries is accompanied by progressively increased exchange over dentine.

10. P^{32}, Ca^{45}, and S^{35} exchange at a great rate in the vicinity of carious lesions.

CONCLUSIONS: The autoradiographic tracing of Sulphur 35 tagged material in sections treated by routine histological technique have revealed that sulphur is retained mainly as sulfopolysaccharides in a variety of structures. An abundant amount of such has been found in the cartilage, bone, dentine, and enamel and is closely related to the phenomenon of mineralization. The preliminary in vitro exchange seems to indicate that this material is present along with the crystal lattice and seems responsible for decrease in P^{32} in vitro exchange of adult mineralized tissues. The calcium 45 tracing of growth in the dentine of incisors of pigs has revealed a pattern of growth comparable to that of the previously described incisor of rat. Sodium fluoride introduced in the diet for 30 days or more in proportions of 200 and 1000 p.p.m. has produced a marked retardation of growth as gauged by mineral deposition and also an irregular wavy pattern of dentine apposition.

APPENDIX "B"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: The effect of topical application of silicones in protecting teeth against acid solutions in vitro

Grantee: Dr. Gordon Nikiforuk

Project No.: DR 48 Amount of Grant: $2,240

SUMMARY OF WORK

The chemical structure of silicones imparts to them a stable oxidized structure common to silica and a thermal stability and resistance to many types of reagents to an extent not found in any of the organic polymers. Their ability to form water repellent surfaces offers theoretical possibilities of preventing corrosion of teeth.

Thirty silicone preparations have been tested for their ability to protect teeth against acid etching in vitro. The most striking protection was offered by a phenyl-methyl and a methyl silicone resin.

Two preparations containing the above resins have been developed. The resins are concentrated by evaporation, partially polymerized by heating and then dissolved in methyl methacrylate monomer. It is suggested that the above preparations have definite clinical uses. Clinical evaluation of the preparations with respect to their usefulness as cavity liners is now in progress.

APPENDIX "B"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: The effect of topical application of silicones in protecting teeth against acid solutions in vitro

Grantee: Dr. Gordon Nikiforuk

Project No.: DR 48 Amount of Grant: $2,240

Introduction

Silicones are polymeric compounds composed of multiples of the structure $(R_2SiO)_x$, where R is the hydro-carbon radical. This chemical structure imparts to silicones a stable oxidized structure common to silica, and since certain R-Si linkages have been shown to be exceedingly inert and resistant to oxidation, it follows that the combination should provide thermal stability and resistance to many types of reagents to an extent not found in any of the organic polymers. The ability of these agents to form water repellent surfaces offers theoretical possibilities of preventing corrosion of teeth.

This report deals with the effect of topical applications of some silicone preparations in protecting teeth against acid solutions in vitro.

Methods and Materials

The silicone polymers and related compounds tested included the following members: the methyl silicone oils; alkyl silicone resins; alkyl-aryl silicone resins; and methylchlorosilanes.

The original screening of some thirty silicone preparations was accomplished by applying the silicone to one-half of the tooth surface, using the other one-half as a control, and immersing the tooth in a 0.5 M. lactate buffer, pH 4.0, for one day. Silicones that protected surfaces against etching were further tested as follows: wax windows of standard dimension (approximately 3 mm. in diameter) were prepared on flat surfaces of non-carious teeth. The surface was then coated with a silicone preparation, dried for ten minutes in air, and then exposed to etching in 45 ml. of a 0.5 M. lactate buffer, pH 4, for six hours.

Identical treatment on another surface of the same tooth formed the control. The degree of etching was assessed by staining with silver nitrate and by measuring the amount of calcium dissolved.

The effectiveness of several silicone preparations in preventing etching of teeth, in vitro, is summarized in Table I. The most striking protection was offered by a group of alkyl-aryl silicone resins and by a methyl silicone resin.

The protection offered by silicone resins and oils against corrosion is due to formation of a thin film. With methylchlorosilane there appears to be a hydrolytic reaction with the methylchlorosilane vapor at the surface of the object being treated, depositing a thin film of methyl-polysiloxane which is a water repellent agent.

The life span of a silicone film when a coated tooth is immersed in boiled saliva for twelve weeks is indicated by the data in Table II.

Methyl methacrylate monomer has been found to be a suitable solvent for most silicone resins.

Application of above findings

A methyl silicone resin (#80) and an alkyl-aryl resin (#804) were selected from the above series on the basis of the following characteristics:

1. Their ability to protect teeth against corrosion
2. Drying time
3. Color
4. Stability
5. Solubility in resin solvent (methyl methacrylate monomer)

Two preparations indicate specific embodiments of the above:

Preparation #1

A methyl-phenyl silicone resin (804) is evaporated in a wide-open vessel at 40°C. until the solid content is 85 per cent. It is then heated to 100°C. for 4 hours to partially polymerize the resin and dissolved in methyl methacrylate resin to make a 30 per cent solution.

Preparation #2

A methyl silicone resin (SR-80) is heated to 100°C. for two hours to partially polymerize the resin. It is then

evaporated until the solid is 65 per cent. This resin is dissolved in methyl methacrylate monomer to make a 30 per cent solution.

It is suggested that these preparations are useful clinically as:

1. Cavity liners under filling materials, particularly under silicate and acrylic fillings.

2. Protective coatings topically applied to teeth prior to the use of orthodontic appliances.

3. Protection for teeth against corrosive agents.

Studies are now underway to determine effectiveness of these preparations for the purposes suggested above.

TABLE I

EFFECT OF TOPICAL APPLICATION OF SILICONES ON ETCHING OF TEETH

TYPE OF SILICONE	Mg. of Ca^{++} dissolved	Mg. of Ca^{++} dissolved in control	Per cent protection
1. Sodium methyl siliconate (2 per cent solution)	0.734	0.772	4.9
2. Dimethyl silicone oils			
A. Viscosity .65	0.536	0.628	14.6
B. Viscosity 350	1.169	.571	0
(i) plus abrasive	1.206	.549	0
C. Viscosity 1000	.800	.360	0
(i) plus abrasive	1.708	.360	0
3. Alkyl and alkyl-aryl resins			
A. Methyl silicone*			
(i) SR #53	.134	.336	61.0
(ii) SR #80	.08	1.42	95.0
B. Alkyl-aryl resins			
(i) SR #98	0.12	0.752	98.4
(ii) DC #801	0.218	0.798	72.7
(iii) DC #803	0.010	1.000	99.0
(iv) DC #804	0.022	0.924	97.6
(v) LC #993	0.012	1.164	99.0
(vi) XR #129G	0.618	0.718	13.9
(vii) DC #2104	.008	.904	99.1
4. Chlorosilanes			
A. Liquid	.466	.408	0
B. Vapours dried for 3½ days	.338	.408	17.1
5. Sodium fluoride (2 per cent)	.896	.912	1.8
6. Sodium silicofluoride (saturated)	.958	1.008	5.0

* Methyl silicone is a generic name of a large number of materials, including oils, resins, and elastomers. The various physical forms it assumes reflect the different molecular complexities of the polymer -- oils are composed chiefly of linear molecules, resins are cross linked, elastomers are superpolymers of high molecular weight and unknown configuration.

TABLE II

ETCHING TESTS ON TEETH COATED WITH SILICONE AND IMMERSED IN BOILED SALIVA FOR TWELVE WEEKS

TYPE OF SILICONE	Mg. of Ca^{++} dissolved	Mg. of Ca^{++} dissolved in control	Per cent Protection
1. Dimethyl silicone oils Viscosity .65	.462	.566	18.3
2. Alkyl-aryl resins			
A. SR-98	.682	1.135	39.9
B. DC-803	.730	.664	0
C. DC-804	.667	.752	11.3
D. DC-993	.797	1.135	29.9
E. XR-129G	.535	.677	20.9
F. DC-2104			

APPENDIX "C"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: Bacteriology of periodontal disease
I. Experimental fusospirochetal infection with mixtures of pure cultures

Grantee: Dr. J.B. Macdonald

Project No.: DR 35 Amount of Grant: $3,610

SUMMARY OF WORK

Recombination mixtures of pure cultures isolated from HG guinea pig exudate have been prepared according to the "wheel-plate" technique. Certain strains were omitted from each recombination mixture. The resultant growth was inoculated into 4 guinea pigs in each instance and passed serially through four to five passages where possible.

Preliminary results indicate that neither the spirochete, the fusiforms, nor any of 6 strains of cocci are essential to the production of typical transmissible infections. One or more members of the Bacteroides strains, the gram-positive rods, and the motile rods appear to be required to maintain typical infection.

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March, 1954

Subject: Bacteriology of periodontal disease
I. Experimental fusospirochetal infections with mixtures of pure cultures

Grantee: Dr. J.B. Macdonald

Project No.: DR 35 Amount of Grant $3,610

The last report dealt with the successful reproduction of fusospirochetal infection with a recombination of 17 pure cultures isolated from a single guinea pig fusospirochetal exudate (HG). The phase of this study to be reported now is the initial stage of attempts to reduce the infective complex to its minimum components.

The technique used has been the 'wheel-plate' technique described previously. The combinations of organisms grown on the wheel-plates are shown in Table I. In Group A the spirochetes were omitted, in B the Gram-positive rods were omitted, in C the cocci were omitted, in D the Bacteroides were omitted, in E the motile rods were omitted, and in F the single strain of fusiform rods was omitted. After incubation each wheel-plate was examined as previously. If unsatisfactory as the result of failure of one or more of the strains to grow or because of contamination, the procedure was repeated until such time as a satisfactory recombination was obtained. Where the recombinations were satisfactory the central mixed growth was emulsified in a tissue grinder and inoculated on to fresh plates as unstreaked drops and incubated anaerobically for 7 days.

The resultant growth in each instance was emulsified in broth and inoculated subcutaneously in 1 ml. dosage into 4 guinea pigs. The animals were examined daily and the character and extent of lesions recorded. Not later than the seventh day following inoculation exudate was aspirated from appropriate lesions from 2 guinea pigs in each group and passed in a dosage of 0.1 ml. to 4 more guinea pigs. When possible this procedure was repeated through 4 or sometimes 5 passages. In certain instances where lesions drained early, additional animals were used.

The results are recorded in Table II. The evidence indicated that either the spirochetes or the six strains of cocci or the fusiforms could be omitted without change in the character of the resultant infection. Lesions in these

three groups were typically "fusospirochetal" except for the character of the flora as revealed by the darkfield microscope. It was clear too that when either the Bacteroides or the motile rods were omitted the lesions were less severe and were no longer serially transmissible. The results are less clear-cut in the group in which the Gram-positive rods were omitted but have been provisionally interpreted as indicating a requirement for one or more of these strains to maintain the typical infection.

This aspect of the study is being continued with repetitions where indicated and omission of various organisms from the recombination mixtures.

TABLE I

RECOMBINATION MIXTURES USED FOR GUINEA PIG INOCULATIONS

	Group A	Group B	Group C	Group D	Group E	Group F
Cocci	JS1 JR4 JR5 K98 K112 JS9	JS1 JR4 JR5 K98 K112 JS9		JS1 JR4 JR5 K98 K112 JS9	JS1 JR4 JR5 K98 K112 JS9	JS1 JR4 JR5 K98 K112 JS9
Gram-Positive Rods	JR3 JB3A JB3B		JR3 JB3A JB3B	JR3 JB3A JB3B	JR3 JB3A JB3B	JR3 JB3A JB3B
Gram-Negative Rods	JR6 K92 K110	JR6 K92 K110	JR6 K92 K110		JR6 K92 K110	JR6 K92 K110
Motile Rods	JV5 K80	JV5 K80	JV5 K80	JV5 K80		JV5 K80
Fusiform	JF5	JF5	JF5	JF5	JF5	
Spirochetes		TMJ3	TMJ3	TMJ3	TMJ3	TMJ3

Table II. Guinea Pig Lesions Resulting from Inoculation of Various Recombination Mixtures

	Passage No.				
	1	2	3	4	5
Group A (No spirochetes)	±	±	++++	+++	
	+	±	++++	++++	
	+	++++		++++	
	++	++++		++++	
Group B (No Gram-positive rods)	±	±	±	0	±
	±	+	+	±	±
	±	++	+	±	
	++++	++	+	++	
		+++			
Group C (No Cocci)	++	+	±	+	
	++	+	+	+	
	++	+	++	++++	
	+++	+++	++++	++++	
Group D (No Gram-Negative rods)	±	0			
	±	0			
	±	0			
	±				
Group E (No Motile Rods)	+	0	±		
	+	0	±		
	+	0			
	++	±			
	++	±			
	+++	+			
Group F (No fusiforms)	0	+	+	0	+++
	+	+	+	0	+++
	+	+	++++	++++	++++
	+	+	++++	++++	++++

Code: 0 = no lesion
± = small nodular lesion, no exudate
+ = localized abscess, less than 1" in diameter
++ = localized abscess, 1" or more in diameter
+++ = spreading infection not fatal in 7 days
++++ = spreading infection fatal in 7 days or less

APPENDIX "D"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: The Cultivation and characteristics of Bacteroides nigrescens

Grantee: Dr. J.B. Macdonald

Project No.: DR 50 Amount of Grant: $400

SUMMARY OF WORK

Filtrate of a culture of Micrococcus aureus promotes growth and pigment production of certain strains of Bacteroides nigrescens. In order to study the nature of this phenomenon a complete synthetic medium has been prepared for Micrococcus aureus. The medium consists of the following:

A. Amino Acids

DL Alphaalanine
DL Valine
DL Leucine
Glycine
DL Proline
DL Aspartic Acid
DL Phenylalanine
DL Tyrosine
L Arginine HCl
DL Histidine HCl
DL Lysine HCl
DL Cystine
DL Tryptophane
DL Methionine

B. Salts

KH_2PO_4

$MgSO_4 \cdot 7H_2O$

$FeSO_4$ $(NH_4)_2SO_4$

$NaNO_3$

C. Vitamins

Thiamin
Nicotinamide

D. CHO

Glucose

Several of the ingredients do not withstand autoclaving and therefore are prepared individually and sterilized by filtration. Preparation of this medium is now completed.

The next step will be the development of a suitable assay method for the 'growth factor' resulting from cultivation of Micrococcus aureus in this medium. The intention is to culture the staphlococci in the synthetic medium, filter the medium, inoculate the filtrate with Bacteroides nigrescens, incubate for a short period, and attempt to quantitate growth with a Beckman spectrophotometer. Following the development of a suitable assay method an attempt will be made to characterize and if possible identify this growth factor.

APPENDIX "E"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: The pathological characteristics of experimental fusospirochetal infections

Grantee: Dr. H.A. Hunter

Project No.: DR 47 Amount of Grant: $2,200

SUMMARY OF WORK

The following report terminates this first phase of the study of the pathological characteristics of fusospirochetal infections.

Eight more rabbits and six rats were given injections intravenously and intraperitoneally respectively of 1.0 ml. and .5 ml. using the supernatant of both thawed and fresh guinea pig exudate.

No pathosis developed in any of the animals.

The results confirm those of Rosebury et al and conflict with those of Graham.

A possible explanation lies in the suggestion that Graham's source material had, by chance, included some pathogenic organisms.

APPENDIX "E"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: The pathological characteristics of experimental fusospirochetal infections.

Grantee: Dr. R.A. Hunter

Project No.: DR 47 Amount of Grant: $2,200

SUMMARY OF WORK

The following report terminates this first phase of the study of the pathological characteristics of fusospirochetal infections.

Eight more rabbits and six rats were given injections intravenously and intraperitoneally respectively of 1.0 ml. and .5 ml. using the supernatant of both thawed and fresh guinea pig exudate.

No pathosis developed in any of the animals.

The results confirm those of Rosebury et al and conflict with those of Graham.

A possible explanation lies in the suggestion that Graham's source material had, by chance, included some pathogenic organisms.

APPENDIX "E"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: The pathological characteristics of experimental fusospirochetal infections

Grantee: Dr. H.A. Hunter

Project No.: DR 47 Amount of Grant: $2,200

The following report terminates this first phase of the study of the pathological characteristics of fusospirochetal infections.

In the October 1953 report, it was shown that fusospirochetal lesions could be produced only by using inocula that contained viable micro-organisms. The injection of filtrates of gingival scrapings and of guinea pig fusospirochetal exudates either subgingivally, subcutaneously, or intravenously in no instance produced signs of disease.

Graham[1] reported that the injection of fresh sterile pooled filtrate of material scraped from the periodontal pockets of patients with 'chronic pyorrhoea' caused injury to the liver and kidneys of animals and concluded that in fifteen of twenty-five animals the toxic material proved fatal. His experiments included:

4 cats, 2 of which died in 4 - 16 days
14 guinea pigs, 8 of which died in 4 - 20 days
5 rats, all of which died in 5 - 7 days
2 rabbits both of which survived, though one passed through a very acute illness.

The demonstrable lesions where present, were fatty degenerations of the liver and/or kidney tubules.

Since the results of this study are in conflict with those of Graham, 6 rats were each injected intraperitoneally with .5 ml. of supernatant from thawed guinea pig exudate. At the end of eight days the animals were apparently healthy, and post-mortem examination showed normal organs and tissues. Again, 6 rabbits were injected intravenously with 1.0 ml. of supernatant of thawed exudate. After 6 days the animals were sacrificed. At no time did they show any recognizable effects, nor were there any histological changes seen. Since it was possible that the process of

freezing for storage may have altered the exudate, two more rabbits received 1.0 ml. intravenous injections of supernatant of fresh guinea pig exudate. After 6 days these two animals were also free of recognizable tissue changes.

The results of the experimental use of filtrates and supernatants of HG[*] guinea pig exudates, either fresh or frozen into cats, rats, rabbits, and guinea pigs, either subgingivally, subcutaneously, intravenously, or intraperitoneally there were no recognizable lesions produced. These results are consistent with those of Rosebury[2] et al, but are in conflict with those of Graham. Since Graham did not include a bacteriological report on his source material, it is not unreasonable that the difference can be explained by suggesting that his material originally contained not only the usual oral bacterial flora, but also some pathogenic organisms capable of elaborating some toxic substance, that were included by change from one or more of the patients' mouths.

[*] For a list of the component micro-organisms of the HG guinea pig exudate, see paper: Macdonald, J.B., R.M. Sutton and M.L. Knoll, The production of fusospirochetal infections in guinea pigs with recombined pure cultures (In preparation)

References:

1. Graham, J.W., Prcc. Roy. Soc. Med. (Odont.) 30:51-58, Nobember, 1936.

2. Rosebury, T., Clark, A.R., Macdonald, J.B. and O'Connell, D.C. Jour. Infect. Dis., 87:234-248, 1950.

APPENDIX "F"

PAPERS PUBLISHED

Committee Paper No.	Title and Author	Reference
1	New aspects of periodontal research, by H.K. BOX	Presented at National Convention of Canadian Dentists, Toronto, 29 May, 1946. (App. "C", 5th Mtg. A.C.D.R., 25 Feb., 1947).
2	Concerning the pathogenesis of periodontal disease, by H.K. BOX	J. Periodontology, 19: 11-20. 1948. (App. "G", 7th Mtg. A.C.D.R., 2 Mar., 1948).
3	The effect of various inorganic salts on the solubility of calcium phosphate, tooth enamel and whole teeth in lactic acid, by J.J. RAE and C.T. CLEGG	J. Dent. Res. 27: 52-53. 1948.
4	Changes in the calcium and phosphate concentrations of saliva and inorganic salt solutions on shaking with calcium phosphate, by J.J. RAE and C.T. CLEGG	J. Dent. Res. 27: 54-57. 1948.
5	An improved method for processing acrylic dentures, by D.R. CHRISTIE	J. Can. Dent. Assoc., 14: 303-317. 1948. (App. "R", 8th Mtg. A.C.D.R., 26 Oct., 1948).
6	The therapeutic properties of periodontal cement packs, by W.J. LINGHORNE and D.C. O'CONNELL	J. Can. Dent. Assoc., 15: 199-205. 1949.
7	Phosphatase in saliva, by J.T. DENTAY and J.J. RAE	J. Dent. Res., Feb. 1949. 4 pp.
8	The treatment of acute oral Vincent's infection, by W.J. LINGHORNE and D.F. STEDMAN	J. Can. Dent. Assoc., July, 1949. 6 pp.
9	A comparison of nine local anaesthetics, by H.S. HAMILTON, B.A. WESTFALL and J.K.W. FERGUSON	J. Pharmacol. Expt. Therap. 94: 299-307. 1948.
10	New methods of repairing acrylic dentures without distortion, by D.R. CHRISTIE	J. Can. Dent. Assoc., 15: 582-590. 1949.
11	The corrosion of cobalt chromium alloys in commercial cleaning agents, by D.E.R. COGGLES, O.J. WALKER and H.R. MACLEAN	J. Can. Dent. Assoc., 16: 75-82. 1950.

APPENDIX "B"

PAPERS PUBLISHED

Committee Paper No.	Title and Author	Reference
1	New aspects of periodontal research, by H.K. BOX	Presented at National Conference of Canadian Dentists, Toronto, 25 May, 1949. (Appr. "C", 5th Mtg., C.D.E., 25 Feb., 1949.)
2	Concentrative petrography in periodontal diagnosis, by H.K. BOX	J. Dent. Res., 27: 11-20. 1948. (Appr. "C", 25th Mtg., 2 Mar., 1948.)
3	The effect of various inorganic salts on the solubility of calcium phosphate, tooth enamel and tricalcium phosphate in lactic acid, by A.J. RAE and C.T. CLEGG	J. Dent. Res., 27: 45-53. 1948.
4	Changes in the calcium and phosphate concentrations of saliva and inorganic salt solutions on shaking with calcium phosphate, by A.J. RAE and C.T. CLEGG	J. Dent. Res. 27: 54-57. 1948.
5	An improved method for processing acrylic dentures, by D.R. CHRISTIE	J. Can. Dent. Assoc., 14: 303-317. 1948. (Appr. "B", 8th Mtg., A.C.D.R., 26 Oct., 1948.)
6	The therapeutic properties of periodontal cement packs, by O.W. HAMMER and D.G. O'CONNELL	J. Can. Dent. Assoc., 15: 199-205. 1949.
7	Phosphatase in saliva, by A.J. RAE and C.T. CLEGG	Can. J. Res., 1949.
8	The treatment of acute oral Vincent's infection, by W.J. LINCHORNE and D.F. STEDMAN	J. Can. Dent. Assoc., July, 1949. 6 pp.
9	A comparison of nine local anaesthetics, by R.B. HAMILTON, B.A. WESTFALL and J.K.W. FERGUSON	J. Pharmacol. Exptl. Therap., 96: 299-307. 1949.
10	New methods of repairing acrylic dentures without distortion, by D.R. CHRISTIE	J. Can. Dent. Assoc., 15: 585-590. 1949.
11	The corrosion of cobalt chromium alloys in commercial cleaning agents, by D.E.R. GOODLESS, O.J. WALKER and H.R. MACLEAN	J. Can. Dent. Assoc., 16: 75-82. 1950.

Committee Paper No.	Title and Author	Reference
12	The relation between buffering capacity, viscosity and lactobacillus count of saliva, by J.J. RAE and C.T. CLEGG	J. Dent. Res. 28: 589-593. 1949.
13	Mineralization of the growing tooth as shown by radiophosphorus autographs (17692), by L.F. BELANGER and C.P. LEBLOND	Proc. Soc. Expt. Biol. Med. 73: 390-391. 1950.
14	Radio-autographic visualization of bone formation in the rat, by C.P. LEBLOND, G.W. WILKINSON, L.F. BELANGER and J. ROBICHON	Am. J. Anat. 86: 2. 1950
15	Fluoride studies related to the human diet, by MARY P. HAM and M. DOREEN SMITH	Can. J. Research, F. 28: 227-233. 1950.
16	Studies in the regeneration and reattachment of supporting structures of the teeth. I. Soft tissue reattachment, by W.J. LINGHORNE and D.C. O'CONNELL	J. Dent. Res. 29: 419-428. 1950.
17	An examination of the fluoride content of teeth from some Ontario districts, by SHIRLEY R. HARNESS and M. DOREEN SMITH	J. Can. Dent. Assoc., Jan., 1951.
18	Uptake of radioactive calcium by the dental tissues of the young rat, by A. D'IORIO and J.P. LUSSIER	Rev. Canadienne de Biol. 10: 175-180. 1951.
19	Relining acrylic dentures without distortion, by D.R. CHRISTIE	J. Can. Dent. Assoc., July,1951.
20	Acrylic fillings - General comments and some experimental data, by D.R. CHRISTIE	J. Can. Dent. Assoc., 17: 427-435. 1951.
21	Ammonia production in saliva, by R.M. BALLANTYNE, C.T. CLEGG, J.J. RAE, and F.H. LAWFORD	J. Dent. Res. 30: 385-387. 1951.
22	Studies in the regeneration and reattachment of supporting structures of the teeth. II. Regeneration of alveolar process, by W.J. LINGHORNE and D.C. O'CONNELL	J. Dent. Res. 30: 604-614. 1951.

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12	The relation between buffering capacity, viscosity and lactobacilli count of saliva, by J.J. RAE and O.T. CLEGG	J. Dent. Res. 28: 589-593. 1949.
13	Mineralization of the growing tooth as shown by radiophosphorus autographs (1952), by L.F. BRIANDER and O.P. LEBLOND	Proc. Soc. Exptl. Biol. Med. 73: 290-291. 1950.
14	Radio-autographic visualization of bone formation in the rat, by O.P. LEBLOND, G.W. WILKINSON, L.F. BRIANDER and J. ROBICHON	Am. J. Anat. 86: 2. 1950
15	Fluoride studies related to the human diet, by MARY P. RAM and M. DOREEN SMITH	Can. J. Research, F. 28: 227-233. 1950.
16	Studies in the regeneration and restablishment of supporting structures of the teeth. I. Soft tissue restablishment, by W.J. LINCHORNE and D.O. O'CONNELL	J. Dent. Res. 29: 419-428. 1950.
17	An examination of the fluoride content of teeth from some Ontario districts, by SHINLEY R. HARDESS and M. DOREEN SMITH	J. Can. Dent. Assoc., Jan., 1951.
18	Uptake of radioactive calcium by the dental tissues of the young rat, by A. D'IORIO and J.P. LUSSIER	Rev. Canadienne de Biol. 10: 175-180. 1951.
19	Relining acrylic dentures without distortion, by D.R. CHRISTIE	J. Can. Dent. Assoc., July, 195
20	Acrylic fillings - General comments and some experimental data, by D.R. CHRISTIE	J. Can. Dent. Assoc., 17: 427-435. 1951.
21	Ammonia production in saliva, by R.M. BRILANTYNE, O.T. CLEGG and J.J. RAE, and F.H. LAWFORD	J. Dent. Res. 30: 385-387. 1951.
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Committee Paper No.	Title and Author	Reference
23	Radioactive isotopes in dental research by J.P. LUSSIER and A. D'IORIO	The Brit. Dent. Annual, 1952.
24	Determination of fluoride in water by the aluminum-hematoxylin method, by MARGARET J. PRICE and O.J. WALKER	Anal. Chem., 24: 1593. 1952.
25	Autoradiographic detection of S^{35} in the membranes of the inner ear of the rat, by L.F. BELANGER	Science, 30 October, 1953. 118: 520-521.
26	The motile non-sporulating anaerobic rods of the oral cavity, by J.B. MACDONALD	University of Toronto Press, Toronto, 1953.
27	Motility in a species of non-flagellated bacteria (20677), by J.B. MACDONALD, M.L. KNOLL and R.M. SUTTON (Introduced by J.K.W. FERGUSON)	Proc. Soc. Exp. Biol. and Med. 1953, vol. 84, 459-462.

The Secretary of the Committee has a small stock of most of the papers listed and will supply copies to members of the Committee on request.

BIBLIOGRAPHY ON CARIES RESEARCH, prepared by Dr. J.S. Bagnall and published by the Committee. This large volume comprising 557 pages, and priced at $10 per copy, contains a thorough review of the literature on caries from 1890 to 1949, collected under 15 main divisions and 130 subdivisions, and a bibliography of 2300 books and articles. Many of these publications were mentioned because they had been referred to by authors whose reports were abstracted and discussed in the review sections. Some 1500 other papers which were examined were not listed in the bibliography because they contained merely expressions of opinion, or duplicated information which had been already summarized from more authentic sources.

Orders for this publication "Bibliography on Caries Research" accompanied by the appropriate remittance should be addressed to: The National Research Council, Ottawa, Canada. Sample pages are obtainable without charge from the Secretary of the Committee, on request.

A SURVEY OF THE LITERATURE ON DENTAL CARIES, publication 225 National Academy of Sciences - National Research Council, Washington, D.C., 567 pages, 1952, is also on file in the office of the Secretary.

March, 1954.

mittee ber No.	Title and Author	Reference
23	Radioactive isotopes in dental research by 1.P. LUSSIER and A. D'IORIO	The Brit. Dent. Annual, 1952.
24	Determination of Fluoride in water by the aluminum-hematoxylin method, by MARGARET 1. PRICE and O.J. WALKER	Anal. Chem., 24: 1593. 1952.
25	Autoradiographic detection of S^{35} in the membranes of the inner ear of the rat, by I.P. BELANGER	Science, 30 October, 1953. 118: 520-521.
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APPENDIX "G"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

AWARD OF SPECIAL TRAVEL GRANTS

1953-54

Name of Grantee	Purpose of Visit	Amount of Award $
Dr. H.A. Hunter, Faculty of Dentistry, University of Toronto, Toronto, Ont.	Attendance at the Annual Meeting of the International Association for Dental Research in Philadelphia, 20-22 March, 1953	150
Dr. C.P. Leblond, Dept. of Anatomy, McGill University, Montreal, Que.	Attendance at the Annual Meeting of the International Association for Dental Research in Philadelphia, 20-22 March, 1953	150
Dr. J.B. Macdonald, Chairman, Division of Dental Research, Faculty of Dentistry, University of Toronto, Toronto, Ont.	Attendance at the Annual Meeting of the International Association for Dental Research in Philadelphia, 20-22 March, 1953	150
Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto, Toronto, Ont.	Attendance at the Annual Meeting of the International Association for Dental Research in Philadelphia, 20-22 March, 1953	150
Dr. G. Nikiforuk, Associate Professor of Pedodontics, Dept. of Dental Research, University of Toronto, Toronto, Ont.	Attendance at the Annual Meeting of the International Association for Dental Research in Philadelphia, 20-22 March, 1953	150
Dr. K.J. Paynter, Faculty of Dentistry, University of Toronto, Toronto, Ont.	Attendance at the Annual Meeting of the International Association for Dental Research in Philadelphia, 20-22 March, 1953	150

Name of Grantee	Purpose of Visit	Amount of Award $
Dr. J.J. Rae, Dept. of Chemistry, Faculty of Dentistry, University of Toronto, Toronto, Ont.	Attendance at the Annual Meeting of the International Association for Dental Research in Philadelphia, 20-22 March, 1953	150
Dr. R.C. Greulich, Dept. of Anatomy, McGill University, Montreal, Que.	Attendance at the Columbus, Ohio, meeting of the American Association of Anatomists, March, 1953	150
Dr. J.B. Macdonald, Chairman, Division of Dental Research, Faculty of Dentistry, University of Toronto, Toronto, Ont.	Attendance at the International Association for Dental Research at French Lick, Indiana, 19-21 March, 1954	150
Dr. J.J. Rae, Dept. of Chemistry, Faculty of Dentistry, University of Toronto, Toronto, Ont.	Attendance at the International Association for Dental Research at French Lick, Indiana, 19-21 March, 1954	150
Dr. G. Nikiforuk, Associate Professor of Pedodontics, Dept. of Dental Research, University of Toronto, Toronto, Ont.	Attendance at the International Association for Dental Research at French Lick, Indiana, 19-21 March, 1954	150
Dr. L.F. Bélanger, Professor of Histology and Embryology, University of Ottawa, Ottawa, Ont.	Attendance at the meeting of the Histochemical Society, Atlantic City, 15,16,17 April, 1954	150
Dr. C.P. Leblond, Dept. of Anatomy, McGill University, Montreal, Que.	Attendance at the meeting of the New York Academy of Sciences, New York, 21-24 January, 1954	150

APPENDIX "H"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: The effect of the endocrines on the teeth and jaws. II. Further studies of the effect of thiouracil on the development of the teeth of rats.

Grantee: Dr. K.J. Paynter

Project No.: DR 38 Amount of Grant: $1000

SUMMARY OF WORK

1. The teeth of young (birth to 36 days) rats, whose mothers received 6-n-propyl-2-thiouracil in drinking water from about the twelfth day of pregnancy at a concentration of 0.1%, were studied.

2. Histologic observations indicated a slowing down of all of the processes involved in tooth formation, similar to that observed and reported under Study 1.

3. At later ages it appeared that eruption had slowed to a more marked degree than other processes, and may have been suppressed altogether. This is possibly related to the marked suppression in bone resorption observed in the thiouracil-treated animals.

4. No differences in size of the fully developed molar crowns was observed between those of controls and experimental animals.

5. It is proposed to suspend this project for the time being and to return the unexpended part of the grant for this year.

APPENDIX "H"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: The effect of the endocrines on the teeth and jaws. II. Further studies of the effect of thiouracil on the development of the teeth of rats.

Grantee: Dr. K.J. Paynter

Project No.: DR 38 Amount of Grant: $1000

It is our opinion that work on this project should be suspended for the time being. It is felt that we have proceeded about as far as we can go using present methods, and lack of time has made it imperative to relinquish some of our activities. Very little of the amount granted for this project during the current year has been spent, and there is, I believe, an amount of some $861.35 to be returned to the Committee.

We have been unable to obtain the soft X-ray pictures of our specimens as planned. The use of the experimental X-ray unit loaned to us by Philips Electric Co., has been delayed by a series of unavoidable and frustrating delays, the latest of which was a break of some nature in the tube itself. The machine has now been returned to Holland for modification.

Employing a technique which we are using in another experiment, we have photographed the upper first molar tooth of each specimen under standard conditions. Measurements made from the photographs were analyzed (control vs. experimental) for differences in tooth size. There was no significant difference between the bucco-lingual diameters of teeth from control animals when compared with those from the thiouracil-treated animals. This confirms the observation of Ziskin, Salmon and Applebaum[1] that the size of rat molar tooth crown remains unaffected by thyro-parathyroidectomy at birth or at seven days.

Histologic observations were presented in our last report and are very similar to those made on teeth of animals which received propyl-thiouracil subcutaneously from birth.[2]

The observations may be summarized as follows:

1. No differences were detected between control and experimental specimens except that the rate of development was considerably retarded in the latter. All processes involved

in tooth formation were retarded in the animals receiving the drug, and, with the exception outlined below, to the same degree, so that what appeared to be normal teeth developed but at a slower rate than normal.

2. In the older specimens eruption of the teeth appeared to be more severely retarded, and, indeed appeared to have ceased altogether. Enamel of teeth formed, and histologically appeared to have become fully mature, while the teeth were still buried within the jaw. The marked delay in eruption is undoubtedly related to the lack of bone resorption which is commonly observed following thiouracil administration.

3. The periodontal membrane appeared narrower in the experimental animals, related no doubt to the lack of function of these teeth.

References: [1] Ziskin, D.E., R.N. Salmon and E. Applebaum, Effect of thyro-parathyroidectomy at birth and at seven days on dental and skeletal development of rats. J. Dent. Res., 19:93, 1940.

[2] Paynter, K.J., The effect of propyl-thiouracil on the development of molar teeth of rats. J. Dent. Res., 1954. (In press)

APPENDIX "I"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

From: Dr. E.E. Johns,
Kingston, Ontario.

On December 14th and 15th the Hooton Collection of the Peabody Museum, Harvard University, Boston, was examined. The collection includes over 4,000 specimens of human crania but the examination was hindered by the fact that much of the material represented disarticulated skeletal fragments, in many cases the mandibles being absent.

Representative specimens of each of the groups were photographed, occlusal, right and left lateral films being exposed. In addition detailed descriptions of the degree of occlusal wear and the state of occlusion was made.

The groups studied were mainly of Indian tribes derived from burial sites in Colorado and Arizona. The following data was obtained and tabulated.

1. Collection number (for purposes of dietary examination)
2. Degree of Wear (according to the Wassel classification)
3. Overbite (recorded in mm.)
4. Overjet (recorded in mm.)
5. Angle classification of occlusion
6. Degree of caries
7. Degree of periodontal involvement
8. Locale
9. Age and sex wherever possible.
10. An estimate aetiology of malocclusion (wherever seen) was attempted.

The following ethnic groups were examined:

Indian skulls from Nashville, Tenn.,
Madisonville, Ohio,
Redwood, Tenn.,
Animas Valley, Colorado,
Yucatan,
Awatobi, Arizona.

Polynesian skulls from the Marquesas Groups and Sandwich Islands
Malay skulls, Malay Peninsula, Thursday Island, North Queensland, Australia
Fiji Islands (Banua Leru)

Martinique
European skulls from Bavaria
Italy
Germany
Yugoslavia
Scandinavian skulls Lapland

One day was spent in the Museum Library and the Widner Library for the purpose of obtaining anthropological data on the following:

1. diet
2. modes of food cultivation
3. food refining methods
4. eating habits.

Much pertinent information was obtained from Dr. E.A. Hooton's text "Indians of the Pecos". Also from Mishkin and Tschopik's "Handbook of the South American Indian, Vol. II." Of special interest was a section from Hooton's text written by Dr. Habib entitled "Notes on the Dentition in relation to the Pecos Collection."

Time was also spent in the Widner Library. However, most of the pertinent material in connection with diet and method of food preparation apparently is to be found in the Museum of Natural History, N.Y.

The writer wishes to express his sincere thanks to Dr. E.A. Hooton and Dr. Herbert Margolis for their co-operation in expediting this investigation.

APPENDIX "J"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: Further studies in the electromyography of the head, face and neck

Grantee: Dr. H. Jenkins

Project No.: DR 37 Amount of Grant: $300

SUMMARY OF WORK

A study of the dentofacial morphology and temporalis muscle activity in normal seven year old children employing cephalostatic radiography and electromyography. Children - 40; Sex - 20 females, 20 males; Age - mean 6 years 8 months Range 5.0 to 7.10.

1. Records collected for each subject:

   9 electromyographic records at one visit
   4 head plates
   dental casts
   diet sheet, height, and weight

2. Data recorded:

   A graphic method of recording an electromyographic abstract was developed for assessment and comparison with other characteristics. Muscle activity was graphed opposite radiographic and model data.

3. Statistical analysis were performed. Correlation tests are not completed at this time.

   (a) Sella nasion/nasion "A" Angle - 80 degrees
   This is similar to Riedels' figure for children 8 - 11 years.

   (b) B and Gn values:
   Gnathion lies on A plane
   B is forward of A plane 1.0 mm.

   (c) Jaw length ratio: $\frac{\text{Condyle } 40 \quad \text{Ptm } 60 \quad \text{A}}{100}$ absolute length

   Maxillary length greater in boys to 1% - probabilitv 10%
   Middle cranial base greater in girls - probability 10%

   (d) Closure path:
   Mean closure path is along A plane
   Range is plus or minus 3 mm.

   (e) Eruption age: Female Male: Similar

5. Electromyography:

(a) Middle temporalis tends to resemble posterior temporalis in behaviour but at a lower amplitude.

(b) Posterior temporalis
28 subjects demonstrated marked activity of this muscle belly. 22 of these were associated with cuspal interference tending to cause posterior mandibular displacement, or an actual marked posterior closure path. (6)

Reference I Riedel

Riedel, R.A. The relation of maxillary structures to cranium in malocclusion and normal occlusion, Angle Orthodontist, July, 1952.

"J-3"

Sample 40 (girls 20, boys 20)

			m	R	s^2	s_m	t
Age							
			6yr 10 mo	5yr 1mo / 7yr 9mo	96.6	2.2	0.9
	Girls						p -
			6yr 7mo	5yr 0mo / 7yr 10mo	119.9	2.5	
	Boys						
			79.95	74.0 / 84.0	8.8	0.65	p - .014
SNA	Girls						
R.A. Riedel Angle Orth. Sample 24 children							
July 1952 Children 8 - 11 Mean 80.79				73.5 /			
Std Dev 3.85	Boys		79.80	85.5	13.3	0.82	
			m	R	s^2	s_m	t
B Value							
				-4.0 /			p -
	Girls		100	4.5	5.3	0.51	0.21
				-3.0 /			
	Boys		0.8	5.0	5.4	0.52	
				-7.0 /			p -
	Girls		-0.1	3.5	8.6	0.66	0.63
Gn value				-4.5 /			
	Boys		-0.6	4.5	5.7	0.54	
Jaw Length			m	R	s^2	s_m	t
				27.5 43.0 / 71.0			
	Girls		32.5 45.1 / 77.05				
				36.5 48.5 / 84.0			
				24.0 44.0 / 72.5			
p- p1%-	Boys		32.1 47.4 / 78.9		10.46 3.34 / 16.87	0.72 0.41 / 0.93	
T 0.39 4.0 / 1.4				39.5 51.5 / 88			
p-							
Jaw Ratio			m	R	s^2	s_m	t
							p 10%
				38.5 51.2			1.72
	Girls	42.1	58.6	43.3 64.8	6.3 9.3	.56 .68	1.70
				32.9 53.4	11.0 8.5	.74 .65	
	Boys	40.5	60.2	45.6 65.1			
				-3 / 3			p -
Closure Path	Girls		-.03		2.60	0.37	0.33
	Boys		0.13	-2.5 2.5	2.30	0.35	1

		m	R	s_R	σ_m	t
Age						
	Girls					
	Boys					
B Value		m	R	s_R	σ_m	t
	Girls					
	Boys					
Gn value						
	Girls					
	Boys					
Jaw Length		m	R	s_R	σ_m	t
	Girls					
	Boys					
Jaw Ratio		m	R	s_R	σ_m	t
	Girls					
	Boys					
Closure Path						
	Girls					
	Boys					

APPENDIX "K"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: An investigation of the mechanism of repair of the supporting structures of the teeth

Grantee: Dr. Charles H. Best

Project No.: DR 22 Amount of Grant: $4200

SUMMARY OF WORK

One of the main objectives in this investigation is to ascertain whether supporting structure of teeth lost during the course of periodontal disease can be regenerated and if so, to develop means of accomplishing this regeneration. In part, our approach to this problem has been to study the desired regenerative process by animal experimentation under more simplified conditions than those encountered clinically (Studies I and II). On the basis of the information so gained, the objective this year was to induce the rapid type of regeneration seen in Study II with conditions more closely resembling the clinical situation in periodontal disease, i.e. following the production of infected epithelialized pockets in dogs. New problems were introduced by the more complex conditions.

Three studies are being carried out to investigate these new problems from different angles. These will be reported in detail at the March meeting of the Committee. The rapid type of regeneration of supporting structure similar to that obtained in Study II has been successfully induced in infected epithelialized pockets in dogs.

The endocrine studies mentioned in the preceding reports are being carried on as time permits. An interesting lesion has been found in the principal fibres of the periodontal membrane in rats. The pathogenesis of this lesion is being studied.

APPENDIX "L"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radioactive elements.

Grantee: Dr. C.P. Leblond

Project No.: DR 23 Amount of Grant: $3,000

SUMMARY OF WORK

It may be recalled (as pointed out in the September 1953 report) that crystals of dentin were deposited mainly at the predentino-dentinal junction as shown by P^{32} and Ca^{45} radioautographs. However, the formation of the matrix was shown (with C^{14} radioautography) to take place in the deeper layers of predentin. It was therefore, presumed that, at the predentino-dentinal junction, some material was added to the predentinal matrix to transform it into dentinal matrix and thus make it suitable for the deposition of mineral crystals.

Histochemical and chemical investigations were carried out by Miss Y. Kumamoto to find out what happens at the predentino-dentinal junction. The histochemical investigations with the periodic acid-Schiff and metachromasia reactions revealed that the neutral polysaccharides detected by the former and the acid polysaccharides detected by the latter were much more abundant in dentin than in predentin. Several explanations of this phenomenon appeared plausible. Either polysaccharides are added at the predentino-dentinal junction (1st hypothesis) or they exist in predentin but in a non-reactive form. The appearance of reactivity at the junction could then be due to an unmasking of reactive groups (2nd hypothesis) or to the addition of reactive radicals (3rd hypothesis.)

The dentin from incisor teeth of very young rats given C^{14} was hydrolyzed on resin and chromatographed on paper. Areas of the paper corresponding to various sugars were eluted and their radioactivity determined. Approximately half of the radioactivity recovered after hydrolysis consisted of sugars while the other half was found adsorbed on the

resin and presumably consisted mainly of amino acids. Since this was true at 11 hours after injection, that is to say, a time when C^{14} is in the predentin, it was concluded that the predentin contains proteins and polysaccharides (revealed by the presence of galactose and small amounts of other sugars).

The low intensity obtained after the periodic acid-Schiff reaction in spite of the presence of sugars such as galactose in predentin is contradictory. The most likely explanation is that the periodic acid reactive (1, 2 glycol) groups of galactose and other sugars present are blocked. These groups of the sugars may become unblocked at the dentino-enamel junction and thus produce the increase in the intensity of the periodic acid-Schiff reaction observed in this region. Thus, the second of the two hypotheses outlined above would account for the abundance of neutral polysaccharides in dentin.

Our data also show the appearance of a small amount of glucuronic acid in predentin, thus indicating that acid polysaccharide may also be present. On the other hand, the results obtained by Bélanger using radio-sulfur autographs show that S^{35} is mainly deposited in the form of an acid polysaccharide at the predentino-dentinal junction (where metachromasia is also maximal). Perhaps the bulk of the acid polysaccharides found in dentin arise from polysaccharides preformed in the predentin and sulfated at the junction, according to the third of the hypotheses presented above.

Once these preliminary hypotheses have been confirmed experiments will be devised to find out if the presence of the neutral or acid mucopolysaccharides (or both) at the predentino-dentinal junction is the directing factor in calcification.

APPENDIX "L"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radioactive elements

Grantee: Dr. C.P. Leblond

Project No.: DR 23 Amount of Grant: $3000

The work carried out since a report was sent to the Associate Committee on Dental Research in September, 1953, may be subdivided into two parts: (1) Preparation of articles and (2) Continuation of research work with emphasis on the organic matrix of dentin.

(1) Publications. Two publications have been nearly completed during the last few months (i) a paper by Y. Kumamoto and C.P. Leblond entitled "Radioautographic visualization of the mineralization of dentin as shown with radioactive calcium". We expect to submit this paper for publication in the Journal of Dental Research.

A review article written in collaboration with Drs. L.F. Bélanger and R.C. Greulich is being prepared for the Annals of the New York Academy of Sciences. This paper presents the detail of data which have been summarized in a conference given before the New York Academy of Sciences. It is an attempt to synthesize the results obtained in the study of the matrix of teeth and bone as shown with C^{14} and S^{35}, and the deposition of mineral elements as shown with Ca^{45} and P^{32}.

Incidentally, the paper entitled "Radioautographic visualization of the formation and fate of the organic matrix of dentin" written in collaboration with Dr. Greulich has been accepted for publication in the Journal of Dental Research (see detailed list).

(2) Formation of the organic matrix in dentin

Experimental work with the radio-elements of calcium and phosphorus have shown that the deposition of the mineral salts takes place essentially at the junction of dentin and predentin. The 18 micra or so of dentin did not contain significant amounts of mineral salts. It was decided therefore to investigate the organic moiety of the predentin and dentin, in the hope that eventually a difference in their composition will give a clue to the mechanism of the calcification taking place in their border.

The first step in the investigation was to re-examine the differences between predentin and dentin using histochemical methods such as the periodic acid-Schiff (PAS), toluidine blue and other stains. In addition, chemical methods were used to determine which substances contribute to the histochemical reactions and were responsible for the differences between the two tissues.

Histochemical Reactions

Materials and Methods

Young rats were killed and their jaws fixed in alcoholic formalin, Orth, Carnoy, Susa and 4% aqueous lead subacetate fixatives. Paraffin sections of these teeth were then stained by different techniques. The two methods which will be considered in some detail are the periodic acid-Schiff (PAS) reaction and toluidine blue stain.

Periodic acid-Schiff (Hotchkiss '48)

After deparaffinization and hydration, the tissues were immersed in periodic acid for 5 minutes, rinsed in 70% alcohol then placed into the Schiff reagent for 15 minutes, which was followed by three rinses in potassium metabisulfite wash water. The slides were then dehydrated through alcohols and mounted in Canada balsam.

Toluidine Blue stain for metachromasia

After deparaffinization and hydration, the tissues were put into a 0.1% aqueous solution of toluidine blue containing 1% ethyl alcohol. Staining was accomplished in 1 minute; longer times did not seem to produce good results. The tissues were rapidly rinsed and dehydrated through acetone, acetone: xylol 1:1, xylol, then mounted in Canada balsam. It was found that 95% alcohol, absolute alcohol, glycerol and dioxane removed the dye.

Other histochemical stains

Other dyes used to stain the teeth sections included Mallory's triple stain for connective tissue (acid fuchsin, aniline blue, orange G), Masson's trichrome stain (Ponceau-acid fuchsin, light green, Weigert's iron hematoxylin), Harris' hematoxylin and eosin, and Von Kossa.

Results

PA-Schiff

The enamel had only a pale almost background tint of pink, while dentin was a deep mauve colour. The reaction in the predentin was much lighter than that of the dentin. The cellular elements of the tooth stained to varying degrees but never as deeply as the dentin. In the pulp, small

globules which were PAS positive were found as in other loose tissues about the tooth.

Toluidine Blue

Toluidine blue stained all structures blue except the dentin which stained a deep purple and predentin, a very light but definitely purple colour. Gradients in the intensity of the metachromatic stain was noted, namely, from the pulpal surface to the dentino-enamel junction. The following changes were noted: light in the predentin, maximal at the predentin-dentin border, and decreasing from this portion to the dentino-enamel junction. The colour was greater in the occlusal than in the basal portions.

Triple stains: Mallory's and Masson's

With these stains, the enamel may be seen in layers corresponding to pre-enamel, young enamel, and transitional and mature forms. A red or a shade thereof characterized young enamel, while transitional and mature enamel are not distinguishable from each other. The dentin shows the uptake of only one dye, aniline blue in the case of Mallory's and light green in the case of Masson's stain. The predentin is much less intensely stained than the dentin.

Harris' hematoxylin and eosin

With the hematoxylin and eosin stains, enamel is coloured red and the dentin purple. The predentin again takes up the stain but to a lesser degree than the dentin.

Von Kossa

The mineralized portion shows up as dark brown-black areas which included the enamel and dentin but not the predentin and the soft tissues.

Conclusion

From these histological staining techniques, a few generalized conclusions may be made. The enamel showed varied intensities of dye uptake depending on the technique use, e.g., little with toluidine blue and PA-Schiff, more with eosin or routine stains. Thus, tentatively, the organic moiety of the enamel may be said to contain more substances reacting to general histological stains and less to specific components such as polysaccharides and chondroitin sulphate. This does not exclude the possibility of the presence of these substances as indeed, some glucosidic carbohydrate has been found in the enamel (Stack '52), but merely to bear the fact that staining reactions are due more to other organic substances.

The dentin in contrast to enamel, does contain more polysaccharides and chondroitin sulphates as well as other

organic substances which combined with the histological dyes. Indeed, chondroitin sulphate has been chemically isolated (Hess and Lee '52) from human dentin and is probably responsible for metachromasia. The predentin although its colour reactions are the same always displayed a lesser uptake of the dyes. It is not known whether this is due to a lesser amount of stainable material or whether the staining reactions are masked by some substance, or are in an unstainable form. Whatever the reason is for the staining reaction of the dentin and predentin there is no doubt that the area about the junction of the dentin and the predentin is the site of some important chemical changes which enables the incorporation and retention of the mineral salts: essentially polysaccharides of two types (periodic acid reactive, metachromatic) appeared at the same time as the ability to calcify.

2. Chemical Analysis

Chemical analysis of the dentin was concentrated on the sugar component since the compounds which stained with PA-Schiff technique was concluded to be due mostly from the sugars. The method of obtaining the carbohydrates from the tissues were developed in this laboratory (Glegg et al, '53; Glegg and Eidinger, unpublished) and consisted of hydrolysis with a resin and the use of paper chromatography to identify the sugars resulting from the hydrolysis. The method at the present stage is qualitative.

A means to isolate the carbohydrates derived from the predentin as those from the dentin were accomplished by the use of labelled carbon techniques. Previous experiments conducted radioautographically in this department (Greulich '53, Ph.D. thesis '54, in press) showed that 4 hours after injection of C^{14}-labelled bicarbonate into very young rats it was deposited in the predentin close to the tips of the odontoblasts, while 24 and 72 hours later it was seen in dentin. Therefore, according to whether the analysis of the organic substance is carried out at early or late time intervals, it will be known whether C^{14}-labelled substances are in predentin or dentin. It was, therefore, decided to investigate the nature of the C^{14}-labelled materials at various time intervals. Two intervals were studied namely, 11 hours after injection to detect the carbohydrates of predentin and 36 hours for those of the dentin.

In this experiment, very young rats were used to minimize the amount of mineral salt in the dentin. Also, it is possible to eliminate much of the enamel when the animals are young. (This was found before hand by extracting incisors from young rats of 9 grams and removing the pulp and the connective tissue surrounding the tooth. The surface of the enamel was visible under the dissection microscope and thus, the enamel could be scratched off except for a small amount at the tip where it was too hard to be scraped off). By staining the processed incisor, it was found that

it consisted of dentin, predentin and a layer of odontoblasts. The molars were removed but attempts have not been made to process them because their cusps make the removal of enamel and pulp very difficult.

The experiments conducted chemically fall into the following groups.

Experiment I

A pilot experiment using rats injected with radiocarbon and sacrificed at 11 hours after injection to determine if there were detectable amounts of carbohydrates and amino-acids in the predentin.

Experiment II

To determine whether carbohydrates from 5 rat incisors (upper and lower), were sufficient to show as spots after spraying the chromatogram with a colour developer.

Experiment III

A study of predentin utilizing 9 rats which were sacrificed 11 hours after C^{14} injection.

Experiment IV

A study of dentin utilizing 10 rats which were sacrificed 36 hours after C^{14} injection.

Materials and Methods

ANIMALS: The animals utilized were young and weighed between 9 and 15 grams at the time of injection. During the course of the experiments, the rats were allowed to be nursed by their mother.

DOSE OF RADIOACTIVITY: Each rat was given 60 μc of C^{14} as bicarbonate titrated with acetic acid to the end point of the indicator, phenolphthalein, which is slightly alkaline.

METHOD OF INJECTION: The animals were injected subcutaneously by inserting a No. 26 gauge hypodermic needle at the back of the neck and passing the needle towards the rump where the C^{14} solution was deposited.

SACRIFICE: The animals were killed under anesthesia and their jaws removed. In experiments I and II the jaws were placed in a covered petri dish saturated with 0.09% saline solution. The petri dish was kept cool by placing it in another larger dish with ice cubes. In experiments III and IV (9-10 rats injected with C^{14}) the jaws were put into a fixing fluid of 1 part 40% neutralized formaldehyde and 3 parts 95% alcohol, as in an ordinary histological technique.

PREPARATION OF SAMPLE

Each incisor was cleaned of adhering bone and tissue under the dissecting microscope. During this process, they were kept from drying with physiological saline solution. Finally, the surface of the enamel and its formative organ were scratched with a dissecting needle and forceps, which removed them except at the very end of the incisal portion, where it was too hard and could not be removed by this method. The tooth was split by passing one blade of a fine pair of scissors into the pulp chamber and cutting it open. The pulp was removed by forceps and fine needle. After flushing the individual pieces of processed teeth they were either immediately dried individually on filter paper (care was taken not to fray the filter paper which would leave bits of it and add carbohydrates) or returned to the fixing fluid for the usual period of fixation before drying in the same manner. The processed teeth consisting mainly of dentin, but also containing predentin and odontoblasts were then weighed. The alcoholic formalin fixed teeth were allowed to sit in the cold room (0-4°C) for a few days to rid it of formalin before proceeding with the analysis.

HYDROLYSIS

The tissues were placed in digestion tubes made from 1/2 inch diameter glass tubing sealed and formed into a small bulb at one end. An amount of Permutit resin (hydrogen form of a polystyrene sulfonic acid resin) equal to 12 times the weight of tissues to be hydrolyzed was also placed in the tube, and about 3 cc of distilled water added. These tubes were then sealed. Hydrolysis was carried out in an oven at 100°C for 2 days. The tubes were occasionally shaken during the hydrolysis.

After completion of hydrolysis, the tubes were removed from the oven, cooled and broken open. The fluid was filtered off through Whatman No. 1 paper. The resin was washed several times with distilled water and the washings filtered and collected. The fluid, containing the carbohydrates was then dried in a vacuum oven at 37°C.

The process to this point was the same for all the 4 experiments (minor differences have already been pointed out). The individual experiments will now be described; however, before doing so, the chromatographic method employed will be described since it is the same throughout for all four experiments.

CHROMATOGRAPHIC METHOD

A large Schleicher and Schuell No. 589 white ribbon paper was cut 35 cm by 42 cm and a line of origin drawn 1 1/2 inches from the edge of the narrow side. After the unknown

samples were placed at the origin, the paper was then stapled into a cylinder 42 cm long, and subjected to the ascending chromatographic method. The medium for chromatography is a pyridine, butanol, water mixture. Each paper was ascended three times. The sites containing the sugars were located by spraying with a reagent, aniline hydrogen oxalate and the colour developed in an oven at 100°C for 5 - 10 minutes. Marker sugars employed to identify unknown samples consisted of the following: glucuronic lactone, galactose, glucose, mannose, fucose, ribose, and glucuronic acid. Previous experiments had shown these sugars to be present in reticulin and its derivatives (Glegg et al, '53).

EXPERIMENT I

The resin was washed successively with 1N and 2N HCl and ether and their washings were collected and dried and counted for radioactivity. The dried carbohydrate residue from the supernatant was then redissolved in a small volume of distilled water and subjected to ascending paper chromatography. Marker sugars were also set up on each side of the teeth sample. After the papers were chromatographed, only the marker sugars were sprayed to show the individual sugars. The processed tooth sample was not sprayed but instead areas corresponding to marker sugars were cut out and eluted with distilled water. The elute was dried and plated for counting in the Q gas flow counter.

EXPERIMENT II

The residue of carbohydrates obtained after drying in the vacuum oven was dissolved in 0.1 cc of distilled water and chromatographed in graded volumes from 3 to 15 lambda. In this experiment, which was of non radioactive teeth, the pulp was subjected to the same treatment. Both the marker and unknown samples were sprayed.

EXPERIMENTS III and IV

Experiments III and IV will be described together here since both were done using identical procedures.

The residue of carbohydrates were dissolved in 0.07 cc of distilled water. Each of the unknown were divided into 2 parts: 40% and 60% of the volume was placed on each origin of the chromatogram, with markers on each side. Carriers of 1/2 the amount on the markers were placed on each of the spots containing the radioactive samples. After chromatographing, only the marker sugars were sprayed with aniline hydrogen oxalate. Areas corresponding to the marker sugars were cut out and eluted from the column containing 40% of the sample. The elutes were counted for radioactivity. The other column containing 60% of the sample, was placed against an X-ray film, carefully wrapped and stored for exposure in a cool place.

RADIOCARBON PRESENT IN DIFFERENT FRACTIONS OF PREDENTIN AND DENTIN (COUNTS/ 100 SEC)

	Experiment I Unfixed tooth (11 hours after C^{14} injection)	Experiment III Fixed tooth (11 hours after C^{14} injection)	Experiment IV Fixed tooth (36 hours after C^{14} injection)
Whole dentin before hydrolysis	2464	2983	1912
SUPERNATANT (Sugars)	357		
Glucuronic lactone	14.6*	0.4	7.3
Ribose		21.3	23.8
Fucose	14.1	1.2	5.4
Mannose	9.4	1.2	5.6
Glucose	9.6	9.7	8.0
Galactose	124.4	60.6	72.0
Unknown	63.5		38.7
Glucuronic acid			5.6
Origin		23.8	31.2
WASHINGS OF RESIN (mainly amino acids)			
1 N HCl	218.0		
2 N HCl	95.4		
Ether	0		

* In Experiment I, the areas corresponding to glucuronic lactone, glucuronic acid and ribose were pooled and gave a count of 14.6 per 100 sec.

Results

The chromatographic analysis of non radioactive teeth (Experiment II) showed the presence of clear-cut spots for galactose, glucose, mannose and fucose in the pulp, while in the dentin, definite spots were observed only for galactose and an unknown, with the possibility of other sugars being present in small amounts.

The results on radioactive samples (Experiments I, III, IV) and pertinent data connected with them are tabulated. Chromatograms are being exposed to X-ray films (radioautography), but it is expected that a long time will be required before results are available. The table shows that C^{14} has been used in the synthesis of monosaccharides, mainly galactose, and amino acids.

Discussion

The isolation of several sugars containing radioactivity from the teeth of rats sacrificed at the early and late time intervals showed that both predentin and dentin do contain these substances.

The possibility of sugars arising from the odontoblasts may be disregarded since previous radioautographic studies showed little or no radioactivity in these cells while the predentin (at early intervals) and the dentin (at late intervals) reacted intensely. Therefore, if radioactive sugars and amino acids are contributed by the presence of odontoblasts, it must be in insignificant amounts.

The low intensity obtained after the periodic acid-Schiff reaction in spite of the presence of sugars such as galactose in predentin is contradictory. The most likely explanation is that the periodic acid reactive (1, 2 glycol) groups of galactose and other sugars present are blocked and therefore unreactive. These groups of the sugars may become unblocked at the dentino-enamel junction and thus produce the increase in the intensity of the periodic acid-Schiff reaction observed at this region. Thus the abundance of neutral polysaccharides capable of reacting with periodic acid in the dentin would be due to the unblocking of 1, 2 glycol groups at the predentino-dentinal junction.

Our data show the appearance of a small amount of glucuronic acid in predentin, thus indicating that acid polysaccharides may also be present. On the other hand, the results obtained by Bélanger using radio-sulphur autographs show that S^{35} is mainly deposited in the form of an acid polysaccharide at the predentino-dentinal junction where metachromasia is also maximal. Perhaps, the bulk of the acid polysaccharides found in dentin arise from polysaccharides preformed in the predentin and sulfated at the junction.

Conclusion

Radioactivity was found in a number of sugars in both the dentin and predentin, which can participate in the periodic acid-Schiff reaction. It is suggested that some change occurs in the sugars at the predentino-dentinal junction resulting in the different degrees of colour intensity after histochemical reactions in the predentin and dentin.

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LEBLOND, C.P., R.E. GLEGG, R.C. GREULICH and Y. KUMAMOTO: Visualization of the formation of the organic and mineral components of the dentin in the tooth of the rat. XIX Internat. Physiol. Cong., 1953, Montreal, Aug. 31 - Sept. 4.

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PRESENTATION AT SCIENTIFIC MEETINGS (concluded)

GREULICH, R.C.: Entry of radiocarbon from labeled bicarbonate into the organic matrix of growing bones and teeth. Anat. Rec., 1953, (Suppl.), 115: 312.

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APPENDIX "M"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: Development of a rapid method for grinding thin sections of plastic-embedded teeth.

Grantee: Benjamin N. Kropp

Project No.: DR 54 Amount of Grant: $820

SUMMARY OF WORK

I. Preparation of teeth for embedding

The great importance of achieving perfect dryness of teeth before plastic embedding is emphasized.

II. Embedding whole teeth in transparent plastic

This deals with the orientation of teeth in the plastic embedding medium, and the plane of sectioning.

III. Cutting plastic-embedded teeth

The basic apparatus developed for this purpose, though incomplete, is described. Some problems encountered in this connection, and our approach to their solution, are outlined.

IV. Grinding and polishing of embedded sections

This follows the method previously used for bone and it is found that teeth require no changes in this method. A holder for use during grinding is described.

APPENDIX "B"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: Development of a rapid method for grinding thin sections of plastic-embedded teeth.

Grantee: Benjamin N. Kropp

Project No.: DR 54 Amount of Grant: $820

SUMMARY OF WORK

I. Preparation of teeth for embedding

The great importance of achieving perfect dryness of teeth before plastic embedding is emphasized.

II. Embedding whole teeth in transparent plastic

This deals with the orientation of teeth in the plastic embedding medium, and the plane of sectioning.

III. Cutting plastic-embedded teeth

The basic apparatus developed for this purpose, though incomplete, is described. Some problems encountered in this connection, and our approach to their solution, are outlined.

IV. Grinding and polishing of embedded sections

This follows the method previously used for bone and it is found that teeth require no changes in this method. A holder for use during grinding is described.

APPENDIX "M"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: Development of a rapid method for grinding thin sections of plastic-embedded teeth.

Grantee: Dr. B.N. Kropp

Project No.: DR 54 Amount of Grant: $820

This work may be reported under the following headings:

I. Preparation for embedding.

II. Embedding whole teeth in transparent plastic.

III. Cutting plastic-embedded teeth.

IV. Grinding and polishing of embedded sections.

The findings reported in my paper "Grinding thin sections of plastic-embedded bone" are basic to the material of this report. Those findings are not repeated here since a copy of the manuscript was filed with my application for this grant.

I. Preparation (of teeth) for embedding

The tooth must be perfectly dry before embedding is attempted. The freshly extracted tooth is cleaned of adherent debris by gently brushing, and it is then washed in running water for 1 to 3 hours. Teeth that have been in formalin or other fixing fluids must be washed for 24 hours. Our method of drying teeth is to place them in an oven at 37°C for 3 days. Although they are then adequately dessicated it does no harm to leave them in longer. Indeed, we store our specimens in this way and remove them from the oven only for embedding.

Failure to completely dry the specimen before embedding results in clouding of the plastic and sometimes cracking of the disc on cooling (Fig. 4). Traces of moisture in the tooth are expressed into the plastic medium by the pressures required during the molding process, and such specimens are worthless for microscopic study. Also moisture cracks in the plastic which appear immediately after removal of the disc from the mold, tend to spread throughout the disc within a few days, and in one case the disc shattered.

II. Embedding whole teeth in transparent plastic

This follows the method previously described for bone, but with specific modifications. The orientation of the tooth within the plastic disc will depend on the intended plane of sectioning. When the desired section must pass longitudinally through crown and roots, the tooth is placed in the mold with the buccal or lingual surface upwards, having in mind that the plane of sectioning, and subsequent grinding and observation, will all coincide with the face of the disc (Fig. 1).

When it is desired to obtain transverse sections of a tooth, the specimen is placed in the mold so as to rest upright on the crown. This can be achieved by first grinding the crown lightly so as to produce several flattened areas. The powdered resin is then placed around the vertical tooth and the disc made by the usual procedure. The disc must, of course, be made tall enough to accommodate the tooth (Fig.2) and a general rule is that each 10 ml of plastic adds approximately 1 cm of height to the disc.

In making the disc with the embedded tooth the general procedure as previously given for bone is followed. However, with teeth best results are obtained if the temperature is not permitted to go above 130°C since at higher temperatures we have found that both enamel and dentin tend to discolour. This discoloration does not appear to be the same as the familiar browning of teeth from "scorching", and it may be possible to look further into this phenomenon at a later time.

III. Cutting plastic-embedded teeth

This phase of the investigation is incomplete. The working plan calls for the design and construction of an apparatus which, at a single operation, will serially section an entire tooth (or specimen of bone) in a desired plane. The photograph (Fig.5) shows part of the apparatus. It consists of a 1/2" shaft, 12" long, driven by a 1/3 h.p. motor having a speed of 3450 rpm. The pulleys provide shaft speeds of 3450 rpm, 4293 rpm, and 4965 rpm. Shaft and motor are bolted to a wood base plate, the motor being bolted so as to permit vertical adjustment. The shaft is ball-bearing mounted and grease plugs are provided at the bearings.

The cutting wheels have proved a time-consuming problem. We are experimenting at the moment with 4" phosphor-bronze wheels, 0.006" thick, 1/2" center hole, in parallel mountings of one to five wheels on the shaft. When more than one wheel is used, 4.8 cm dividers, 1 mm thick, are placed between adjacent wheels. The cutting edge is provided by diamond powder peened into the wheels. This has not proved satisfactory because the cost is too great for wide application of the method, and better methods of loading the wheels with the abrasive powder need to be worked out. We are attempting to solve

these problems on the basis of the following alternatives:

1. The use of an abrasive other than diamond powder, but one which approaches it in hardness, which can be standardized as to grit size, and which is substantially lower in price.

2. The use of an alloy capable of cutting enamel, provided it can be made into cut-off wheels or powdered to specific grit sizes for application to cut-off wheels.

In connection with this portion of the work, the following devices are in process of design:

1. A device for holding the plastic-embedded specimen during sectioning.

2. A device for advancing the specimen by measured intervals when it is desired to cut but one section at a time.

3. A device for cooling the specimen during cutting.

IV. Grinding and polishing of embedded sections

To hold the plastic disc during the grinding and polishing process, a simple holder was designed and made. It consists of a 7/16" long section of brass tubing 1-1/16" O.D., 15/16" I.D. and 1/16" wall thickness (Fig. 3). Inside the tube a flange 3/32" long was cut, leaving a horizontal shoulder about 1/32" wide. On making a short vertical cut in the flange, and then a horizontal one above it about 1" long, a spring of sufficient tension is obtained to hold the disc firmly. The holder is high enough to provide a good grip (Fig. 6), and the operator can easily observe the progress of grinding and polishing through the transparent disc from above. If desired, a thin strip of tape may be applied around the top of the holder to provide a "non-slip" grip. In order to grind discs less than 3/32" thick, a small brass ring was made to slip easily inside the flange of the holder before the disc is inserted. The grinding and polishing of teeth was found to require no essential modification of the process described for bone.

APPENDIX "N"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: Study of human dental pulp blood vessels by X-ray micro-arteriographic methods

Grantee: Dr. R.L.de C.H. Saunders

Project No.: DR 51 Amount of Grant: $1200

Since the interim report (Report No. 1) made on September 12, 1953, a number of teeth, of various age groups and obtained from the clinic, have been injected with a sodium citrate-India ink solution containing various radiopaque agents, and decalcified in the manner of the technique outlined in that report. Following this the teeth were sectioned in a freezing microtome and examined to determine their suitability for further microscopic or radiographic studies. Most of these proved unsatisfactory to our purpose, and as yet unfortunately we cannot anticipate nor determine with certainty the factors responsible for lack of uniformity in vascular filling.

Some of the material has however fully met our requirements and photographs of same are enclosed with this report since they demonstrate the possibilities of the technique. The tooth in question was a lower right cuspid, free of infection and decay, obtained from a 51-year old male. The injectant used consisted of Thorotrast (20%), Sodium Citrate (3.8%), and two-thirds strength India Ink diluted with Distilled Water and Sodium Citrate to give a weakly tinted solution (3.8%). Decalcification was effected by means of an ionic bone decalcifier, and completed with Formic Acid (10%). After freezing and sectioning, the section was photographed stereoscopically in segments and reconstructed following a method developed in this department.

The enlargement (Figure 1) conveys a sense of depth and demonstrates the general vascular pattern of the dental pulp as seen in this cuspid; further enlargements are being made for study purposes. On the other hand the stereoscopic plate (Figure 2) provides a good three dimensional view of the vascular tree and its interconnections, as well as its relations to the walls of the pulp cavity and root canal. (The latter figure should of course be studied under a stereoscope; this print has been made in such proportions that it can be reproduced at the same magnification). The vascular pattern so illustrated by Figures 1 and 2 agrees in the main with those features demonstrated photographically in the previous report. The details

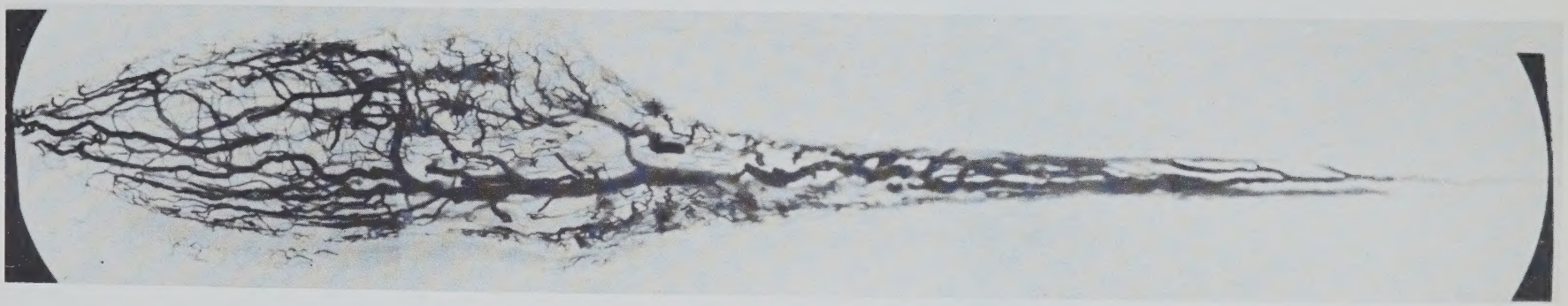
and significance of this pattern are now being studied, by combining the microscopic, stereo-photographic, and micro-radiographic techniques.

In recent months an improved microradiographic technique has been developed in connection with the vascular pattern in certain other tissues, and capillary definition is now obtainable in those tissues. An emulsion sensitized with mercury vapour is being employed, and experiments conducted with various radiopaque agents. Special attention is being paid to the particle size of the injectant, and a radiopaque solution with an average particle size below 0.5 micron and therefore capillary diameter is now being tested for its suitability. This aspect of the work has had to await the development of a satisfactory injection technique, and although it would be premature to make a statement at this time it is hoped that it may yield features not disclosed by the other techniques.

Figure 1. (Examine with hand lens).

Vascular pattern of the dental pulp as seen in the pulp cavity and root canal of a lower right cuspid obtained from a 51 year old man. The tooth was free of infection and decay. Injectant consisted of Thorotrast, Sodium Citrate, and India Ink.

Please return to Prof. R.L. de C.H. Saunders, Anatomy Dept., Dalhousie University, Halifax. N.S.



Legend below
Centre here in stereoscope. →

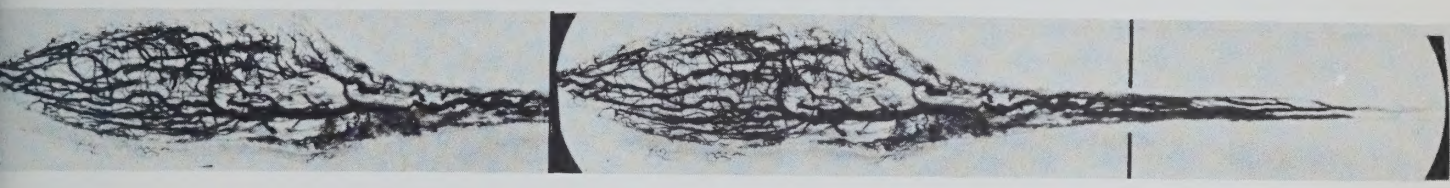


Figure 2. (Examine with stereoscope).

Segmental stereoscopic reconstruction derived from ten photographic exposures, showing the vascular pattern of the pulp cavity and root canal of a lower right cuspid extracted from a 51-year old man.
Injectant: Thorotrast, Sodium Citrate, India Ink.

APPENDIX "O"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: Chemical Mechanisms in Dental Caries

Grantee: Dr. J.J. Rae

Project No.: DR 1 Amount of Grant: $1080

SUMMARY OF WORK

The work during the past year has not progressed as rapidly as was hoped. Experiments designed to conclude a certain section of the work had the annoying habit of yielding ambiguous results which led to more experiments.

(1) The work on ammonia production in saliva, as far as we are concerned is concluded and a third paper embodying the later results of this research will soon be sent to the Journal of Dental Research. A brief statement embodying the salient features of the results obtained can be made as follows:

There is in saliva an ammonia producing mechanism and when salivas are incubated for 24 hours at 37° considerable amounts of ammonia are produced.

This ammonia producing mechanism has the following attributes:

(a) Its activity is due to bacteria.
(b) It is inhibited by fluoride.
(c) It is inhibited by glucose.
(d) Its optimum pH is 6.5.
(e) Its activity is unrelated to l.b.a. counts.

There is in saliva an active urease which liberates ammonia when urea and saliva are incubated. The addition of glucose to this system enhances ammonia production.

(2) The work on the bactericidal material is continuing but yields at times discouraging results. Work of this kind is tedious since time consuming step-by-step determinations of bactericidal activity must be made.

In some cases promising precipitates were prepared only to find that they had no bactericidal properties. Eli Lilly have tested some of the products of this research and recently it has been decided to send promising samples to Dr. R.S. Manly to use in his Inhibitor Survey Research at Tufts College Dental School.

APPENDIX "O"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: Chemical mechanisms in dental caries

Grantee: Dr. J.J. Rae

Project No.: DR 1 Amount of Grant: $1080

The work done during the period March 1953 - March 1954, will be briefly described under two subheadings.

(1) Ammonia Production in Saliva

Two papers on Ammonia Production have been published:-

1. "Ammonia Production in Saliva", J.D.R., Vol. 30, page 385, 1951.

2. "Ammonia Production and Urease Activity in Saliva", J.D.R., Vol. 31, page 281, 1952.

In these papers, it was shown that there is an active Ammonia-producing mechanism in saliva which was inhibited by the addition of glucose. The addition of urea increased ammonia production many times, showing the presence of an active urease in saliva. The addition of glucose to these urea-saliva mixtures increased ammonia production over that observed when no glucose was present. These papers also showed the activity if the ammonia-producing mechanism was unrelated to Caries activity as shown by lactobacillus counts. No relation was observed either between urease content of saliva and l.b.a. counts. That the ammonia-producing mechanism is dependent on the bacteria in the saliva, was shown by the lack of ammonia production by Seitz filtering the saliva.

In an attempt to answer these questions:-

1. "Is the inhibitory action of glucose due to a lowering of the pH below the optimum for ammonia production caused by acid production from the added glucose?"

2. "Is the stimulating effect of glucose on urea-saliva mixtures also due to the acids produced by bacterial action on glucose preventing the pH from going above the optimum for ammonia production?"

The following experiments were performed:

The optimum pH for ammonia production was found to be 6.5. The optimum pH for ammonia production with urea added to

saliva was 7.0.

If, at the same pH, (buffered), ammonia production is less in the presence of glucose, this would indicate that the inhibitory effect of glucose was not attributable to a drop in pH. Difficulties were encountered in maintaining a constant pH. Too high a concentration of buffer interfered with ammonia production, and if shorter times were used to maintain a constant pH, insignificant differences in ammonia were observed.

The results (Nos. 1 and 2) shown below indicate that although the ammonia values were lower than those observed in the absence of buffer, since the pH was kept relatively constant, the inhibiting effect of glucose was still observable.

TABLE NO. 1

Buffer (0.4M) = sodium phosphate-citric acid (0.2M) solution.

No.	Saliva	Buffer	20% Glucose	15% Urea	pH Start	pH End	mg % NH_3
1	2 ml.	4 ml.	--	--	7.00	6.85	15
2	2 ml.	4 ml.	1 ml.	--	7.00	6.50	6
3	2 ml.	4 ml.	--	1 ml.	7.00	7.10	118
4	2 ml.	4 ml.		1 ml.	7.00	7.05	234

Apparently, the answer to our first question is that although the inhibition of glucose on ammonia production may in part be due to the production of acid, when the pH is controlled there is still a noticeable inhibition.

In the table shown above (Nos. 3 and 4), when the pH was controlled, there was still a twofold increase in ammonia production caused by the addition of glucose to the urea-saliva mixtures. This would indicate that the answer to question No. 2 is also in the negative.

That differences noted above might be due to differences in osmotic pressure was investigated by comparing the ammonia production of saliva-and-water and saliva-and-isotonic salt-solution. There was no difference in the ammonia produced.

(2) An inhibiting substance developed when ground, human tooth enamel is shaken with bromoform

A summarized statement of the information obtained to date under this heading is as follows:

When ground, human tooth enamel is shaken with bromoform for one hour, then dried at 160° for 24 hours, the treated tooth enamel contains a substance which inhibits the growth of lactobacillus acidopholus (l.b.a.). As little as 10 mg. of the treated enamel dropped into 4 ml. of a 48-hour broth culture of l.b.a. kills all the bacteria in 24 hours.

Treatment of dentine, calcium phosphate and bone meal under similar conditions yielded materials which had no inhibiting action. Bromoform itself (.125%) did not inhibit l.b.a. growth. Chloroform, when substituted for bromoform produced an inactive material. These facts made us reasonably certain that the inhibition was not due to poisoning by organic solvents ($CHBr_3$ or $CHCl_3$) nor due to calcium or phosphate ions.

Recently, it has been found that bromine dissolved in chloroform (10%) when shaken with tooth enamel produces the inhibitory material and this less expensive treatment has been used to produce the inhibitory substance.

The inhibiting substance can be extracted from the treated tooth enamel with ether, acetone, ethanol and methanol.

Various procedures have been used in an attempt to obtain a pure active material from the methanol extract. Evaporation to dryness yielded a material which was soluble in ether and acetone and was found by qualitative tests to contain chloride, but no sulphur, phosphorus or nitrogen. A fluorescein test for the presence of bromine on the methanol extract of the chloroform-bromine treated tooth enamel gave a negative result.

(a) Three hundred mg. of residue from a methanol extract was dissolved in water and centrifuged. The centrifugate was decanted and 6 ml. of 4% ammonium oxalate solution added. A very small amount (about 2 mg.) of precipitate formed, showing that very little calcium or magnesium is present in the inhibiting substance.

(b) Three hundred mg. of residue from a methanol extract was dissolved in 10% nitric acid. Fifty ml. of a 10% barium nitrate solution was added to the solution. A heavy white crystalline precipitate formed. When the precipitate and the filtrate were separated, put in solution, and brought to the right pH for testing against l.b.a., it was found that there was no inhibitory activity in either fraction. A separate test was made to find out if the 10% nitric acid was exerting a destructive effect on the inhibiting substance and it was found that the nitric acid solution of the residue from the

methanol extract was active in inhibiting l.b.a. growth. This aspect of the work is being pursued further.

(c) 2.521 gm. of ground tooth substance was placed in a platinum crucible and ashed by heating to 900°C for 3 hours. Loss of weight was 0.3173 gm. indicating 12.5% organic material. The ash was transferred to a test tube and shaken with 10 ml. of bromoform for 1 hour. An equal weight of ground tooth substance was shaken with bromoform at the same time. After drying, 50 mg. of each powder was added to a 72-hour l.b.a. broth culture. Twenty four hours later the culture tube containing the ashed tooth substance gave a count of one million whereas the regular tooth substance gave a count of zero. Ashing of tooth substance removes from it the property of combining with bromoform to form an inhibiting substance.

An attempt to purify the inhibiting substance by precipitating the chloride was made. It was found that the chloride-free solution inhibited the growth of l.b.a. But, when comparison was made between the chloride-free solution and the ordinary solution, of inhibiting substance, it was found that the chloride-free solution required a much greater weight of solids per ml. than the ordinary solution to affect inhibition of l.b.a. growth.

A sample of bromoform-treated tooth enamel, an alcoholic extract of treated tooth enamel, and 4 mg. of a precipitate obtained by adding NaOH to the alcoholic extract was forwarded to Eli Lilly and Company for testing against other micro-organisms. The enclosed letter was received in reply (Exhibit I). As stated in the letter, the inhibiting substance seems to have a broad-spectrum of inhibitory effect at quite low concentrations.

Our attempts to identify this substance are continuing.

EXHIBIT I (APP."O")

THE LILLY RESEARCH LABORATORIES

Eli Lilly and Company

Indianapolis 6, Ind., U.S.A.

October 3, 1951

Dr. Charles T. Clegg
Dr. James J. Rae
Department of Chemistry
University of Toronto
Toronto 5, Canada

Gentlemen:

Several months ago you addressed a letter to Eli Lilly and Company regarding a problem on ground tooth enamel and various extracts of this material. Dr. Kleiderer, who replied to your letter, turned over the various material to me for tests. Several of my associates in microbiological research have taken a look at this material and I would like to give you the following report covering the data we have obtained thus far.

Sample a - $CHCl_3$ - treated tooth enamel

This material was insoluble in water and ethanol (although the Toronto letter seemed to imply solubility in ethanol), and was finally dissolved in dilute HCl. The solution was inactive against two species of lactobacilli at 50 mcg. per ml. in broth and against 15 other test organisms, including representative Gram-positive and -negative bacteria and fungi, at levels as high as 100 mcg. per ml. in agar.

Sample b - Alcoholic solution of $CHCl_3$ residue

Sample b inhibited Lactobacillus casei (but not L. leichmannii) at dilutions as high as 1/200 in a broth test. In an agar dilution spectrum test against 15 organisms, the following results were obtained (data are reported on agar dilution units per ml. of solution, based on readings made at 24 and 48 hours):

. . . 2

TO: Dr. Charles T. Clegg
Dr. James J. Rae

October 3, 1951

Test Organism	Agar Dilution Units/ml. 24 Hrs.	48 Hrs.
Staphylococcus aureus	80	<20
Staphylococcus albus	40	<20
Bacillus subtilis	80	40
Mycobacterium phlei	80	40
Mycobacterium tuberculosis(607)	--	40
Mycobacterium avium	80	40
Mycobacterium smegmatis	40	<20
Escherichia coli	20	<20
Proteus vulgaris	20	20
Pseudomonas aeruginosa	20	20
Aerobacter aerogenes	40	20
Klebsiella pneumoniae	40	20
Saccharomyces pastorianus	20	<20
Candida albicans	20	<20
Trichophyton rubrum	--	20

Sample c - Precipitate obtained by adding NaOH to alcoholic solution b.

This entire sample (4.2 mg.) was extracted with acidic ethanol (50%) and found to have slight activity against L. casei in a broth dilution test. L. casei was inhibited by the amount of material extracted from 100 mcg. of the original solids. There was not enough material for a broad screening test.

In summary, samples a and c showed slight or negligible activity in our tests. The solubility properties of these samples were not as we expected from the data obtained in your letter and, of course, it is possible that our extraction procedures may have destroyed the active material.

Sample b has a broad spectrum of activity, the importance of which depends on the purity of the material. The solids in this solution were roughly 830 mcg./ml., which means L. casei was inhibited by about 4 mcg./ml., and the other test organisms by 20-40 mcg./ml.

One of our biochemical boys attempted to duplicate the procedure on the tooth enamel as outlined in your letter. Either he was unable to perform the extractions according to the method which you outlined or else the tooth enamel didn't contain the activity. The procedure he used was as follows:

Seven grams of tooth enamel shaken for three hours at room temperature with 200 ml. of acetone. This was repeated. The acetone extracts were combined, filtered and concentrated in vacuo to dryness. The solids were washed with chloroform (3-20 min. shaking using 200 ml. of $CHCl_3$ for each wash). Then the residue was taken up in 50 ml. of alcohol.

. . . 3

TO: Dr. Charles T. Clegg
Dr. James J. Rae

October 3, 1951

Neither this alcohol solution nor the chloroform solution when taken to dryness and dissolved in alcohol showed any antibacterial activity.

We noted that after the chloroform extraction the remaining material was not very soluble in alcohol.

It would appear that sample b has antibiotic properties and, if it could be purified and not be too toxic, it might be of interest.

We would appreciate your comments on these results and any other data which you might have obtained in the interim on this material.

Sincerely yours,

ELI LILLY AND COMPANY

(sgd.) J.A. Leighty,
Associate Executive Director
Research and Control

APPENDIX "P"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: Electropotentials caused by dental alloys

Grantees: Drs. S.G. Davis and H.R. MacLean

Project No.: DR 43 Amount of Grant: $1025

SUMMARY OF WORK

No work was done on this project from September to January. During this term, January to April, Mr. D. Hetherington is working on the project.

Measurements are being made between the various alloys and the saturated calomel cell, as well as between the alloys themselves. Some difficulty is still being encountered in obtaining consistent data. It is thought that this is due to polarization of the alloys. The effect of polarization is being studied by measuring the charge in potential with time after a known quantity of electricity has flowed. A freshly polished surface is used at the start. The electrometer used to measure potential only requires about 10^{-12} amperes and so the quantity of current drawn during the measurement is not significant.

The measurements are being made with the alloys in contact with Ringer's solution.

APPENDIX "I"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

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APPENDIX "Q"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: Fluoride content of dentin, enamel and alveolar processes of teeth from Sarnia, Brantford and Stratford

Grantee: Dr. M. Doreen Smith

Project No.: DR 13 Amount of Grant: $1625

SUMMARY OF WORK

A number of permanent teeth from Sarnia (no fluorine in water), Stratford (1 p.p.m. fluorine present naturally) and fifteen permanent and 15 deciduous teeth from Brantford (1 p.p.m. fluorine added to water) have been analyzed for fluorine and the results presented in a past report. This report is primarily limited to the Brantford teeth, both primary and secondary which have been coming in the last year and which present considerable interest since length of exposure to artificial fluoridation of water can be observed.

The teeth have been arranged according to age into two groups for both deciduous and permanent teeth. Teeth exposed to fluorine three years or less are in group I and those exposed approximately 7.5 years in group II. For the deciduous teeth Group II has been subdivided into those from subjects under eight years of age and those from subjects eight years and older.

Deciduous teeth (30) from Brantford.

The enamel from deciduous teeth exposed to fluoridated water approximately 7½ years from children under eight years of age shows an appreciably higher mean (0.0118% fluorine) than those from children exposed the same length of time but eight years of age or older (0.0076%) and than those from children 5 - 17 years exposed approximately three years (0.0082%).

The dentin shows the highest mean in the group under eight years exposed for the longer period, the next highest is in the group exposed for the same period but eight years of age or older and the lowest in the group exposed for the short time period. The results for above are presented in three tables.

Permanent teeth (49) from Brantford.

In a comparison of the fluorine content of the enamel in the two exposure groups, the mean is higher in the group

with the longer exposure and the mean for the dentin in the same group is over twice as high as that for the dentin in the group with the shorter exposure. The results for these are presented in two tables.

The rate of gain of fluorine per year of exposure in dentin and enamel of the permanent teeth from Brantford closely approximates that of the rates of gain of fluorine in the teeth from Stratford. A graph is presented to demonstrate this and also a bar graph to compare Brantford results with those from Sarnia and Stratford.

All selection of teeth for analysis was made at random.

APPENDIX "Q"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: Fluoride content of dentin, enamel and alveolar processes of teeth from Sarnia, Brantford and Stratford.

Grantee: Dr. M. Doreen Smith

Project No.: DR 13 Amount of Grant: $1625

The fluoridation of the Brantford water supply was commenced in June, 1945. This presents an opportunity to examine the fluorine content of the enamel and dentin of both deciduous and permanent teeth extracted from individuals residing in the area and to compare results with similar data from Sarnia (no fluorine in water) and Stratford (1 p.p.m. fluorine in water). It is of particular interest to make continued study of Brantford material to learn what effect, if any, the varying time of exposure might have on the different tooth fractions.

No teeth have been received from Stratford this year and only two from Sarnia. The 1953 report gives an idea of the fluorine composition of teeth in these two areas which should be more or less constant. As yet no alveolar processes have been received for analysis.

Deciduous Teeth - Brantford

Fifty deciduous teeth have been separated into dentin and enamel and a chemical analysis of the fluorine content obtained by the methods given in previous reports (1,2). These have been classified according to length of exposure into two groups: those exposed for approximately three years and those exposed for approximately 7 years, 6 months. No teeth were received between these two periods which accounts for the gap of almost four years. In the first group fifteen teeth are arranged according to the age of the subjects from whom they were extracted. Since it would be expected that all of these teeth were erupted before fluoridation of the water was commenced, the results are gathered together in Table I. In the second period of exposure however, the teeth of children seven years or under would most likely have been formed after fluoridation had started. These results are grouped in Table II, and the results of teeth from children eight years and older in Table III, since it is assumed that their teeth have been formed before fluoridation commenced.

APPENDIX "G"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: Fluoride content of dentin, enamel and alveolar processes of teeth from Sarnia, Brantford and Stratford.

Grantee: Dr. M. Doreen Smith

Project No.: DR 13 Amount of Grant: $1625

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Deciduous Enamel

On comparison of the means for percentage fluorine of the enamel in Tables I, II and III, it is evident that exposure to fluoridated water has no effect except on the enamel of teeth formed after fluoridation commenced. The mean for this group is 0.011(8)% as compared with 0.0082% for teeth exposed approximately three years and 0.0076% for teeth of children eight years and older exposed approximately 7.5 years. Table I shows that the mean for children with three years exposure under eight years of age is the same as for those eight years and older. Apparently enamel of deciduous teeth does not absorb fluorine from water after eruption.

Deciduous Dentin

Comparison of the dentin in the same groups shows a mean of 0.030(8)% (Table II) for teeth erupted after fluoridation had commenced whereas those erupted prior to fluoridation, even though exposure time was the same, showed a mean of only 0.025(6)% (Table III). This mean however, is appreciably higher than that for teeth in any age group studied which has had exposure for the much shorter time period, 0.012(6)% (Table I). Table I also shows that the mean for dentin in children under eight years of age is the same as for those eight years and older, in the same age group.

Permanent Teeth - Brantford

Forty-nine permanent teeth from Brantford have been divided into two groups similar to those for the deciduous teeth. Fluorine assays on dentin and enamel of each group have been carried out. In Table IV the results of fifteen teeth extracted after approximately three years exposure to fluorine are given and in Table V are the results of 34 teeth extracted after approximately 7.5 years exposure to fluorine.

On comparison of the enamel in the two groups the mean is higher in the group with the longer exposure. Omitting the one figure abnormally high in the age group "14", it can be seen that the age mean for the six 9 year-old cases exposed 7.5 years is higher than for any other age group in either Table IV or Table V.

A comparison of the dentin of the two groups shows a mean for the one with longer exposure which is over twice as great as that with exposure to fluorine of three years or less. When age means are examined, years of exposure not age of subject appears to be the significant factor.

It is interesting to compare the rate of gain of fluorine per year of exposure for dentin and enamel in each group. These rates are demonstrated in Figure I. The results of the Stratford teeth analyses (reported to the Committee, March, 1953) are also included. Years of exposure are plotted against percentage fluorine in dentin and enamel of Brantford and Stratford teeth. Assuming the years of exposure for permanent Stratford teeth would begin on the average around six years of age, the figure six was subtracted from the ages of the Stratford cases to get years of exposure on a comparable scale to that of Brantford. All the Brantford teeth are in the age range of 9 - 17 years, the forty Stratford teeth are in the age range of 11 - 38 years.

Regression coefficients have not been worked out yet for the figures used in the graph, but the slopes obtained by plotting the average figures show some correlation. The rate of gain of fluorine per year of exposure by dentin in Brantford teeth appears to be about 0.004% in contrast to the 0.001%[x]. Both lines for Stratford are taken to the origin since the initial exposure to fluoridated water commenced with formation of these Stratford teeth. The graphs for Brantford teeth cannot be taken through the origin since at "0" exposure to fluoridated water they may have been exposed from 2 to 14 years to the ordinary sources of fluorine other than water in the Brantford area. A correction for this lowers the Brantford graphs so that they almost coincide with those of Stratford. It can be observed on Figure I that the averages from Stratford teeth for the enamel fluorine for each year show more irregularity than those for the dentin.

Bar graphs for the control area of Sarnia and for Brantford and Stratford are presented in Figure II. The effect of exposure to fluoridated water is apparent especially on the dentin. This is shown also by the D/E ratios of the three areas which are as follows: Sarnia - 2.7; Brantford (3 years exposure) - 2.7; Brantford (7 years exposure) - 3.6; and Stratford - 3.5.

It should be noted that all teeth have been analyzed at random with no sorting as to age, place of origin, permanent or deciduous nature. Several operators have worked on the material simply recording data for each tooth as obtained. Only after a number of results have been handed in has any attempt been made to study them. Usually the operators have not been associated with this part of the work. This year however, the writer would like to acknowledge the helpful assistance of Miss Tung-Yu Lin on the preparation of the tables and charts.

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[x] This relationship may not be a simple linear regression.

References

1. Association of Official Agricultural Chemists, Official and Tentative Methods of Analysis, 1950.

2. Manly, R.S. and Hodge, H.C., Journal of Dental Research 18: 133, 1939.

References

1. Association of Official Agricultural Chemists, Official and Tentative Methods of Analysis, 1930.

2. Manly, R.S. and Hodge, H.C., Journal of Dental Research 18: 133, 1939.

TABLE I

Deciduous Teeth - Three Years Exposure

Age (Yrs)	Type of Tooth	% Fluorine in Dentin	Age Mean	% Fluorine in Enamel	Age Mean	D/E
5	Molar	.0202	.0202	.0222	.0222	0.9
6	Molar	.0093		.0067		1.4
6	Molar	.0188		.0102		1.8
6	Molar	.0096		.0011		8.7
6	Molar	.0112		---		--
			.0122		.0060	
7	Molar	.0074		.0039		1.9
7	Cuspid	.0046		.0018		2.6
7	Molar	.0181		.0124		1.5
			.0100		.0060	
Mean under 8 years		.0124		.0083		
8	Molar	.0178		.0054		3.3
8	Molar	.0144		,0024		6.0
8	Molar	.0113		.0042		2.7
			.0145		.0040	
9	Molar	.0120	.0120	.0085	.0085	1.4
10	Molar	.0096		.0135		.7
10	Molar	.0143		.0154		.9
			.0120		.0150	
12	Molar	.0102	.0102	.0075	.0075	1.4
Mean over 8 years		.0128		.0081		
Mean Value of above teeth		.0126		.0082		2.5

TABLE II

Deciduous Teeth - 7.5 years exposure (under 8 years)

Age (Yrs)	Type of Tooth	% Fluorine in Dentin	Age Mean	% Fluorine in Enamel	Age Mean	D/E
5	Molar	.0478		.0186		2.57
5	"	.0211		.0033		6.39
5	"	.0245		.0080		3.06
			.0311		.0099	
6	"	.0343		.0153		2.24
6	"	.0334		.0115		2.90
6	"	.0252		.0109		2.31
			.0310		.0126	
7	Molar	.0228		.0084		2.71
7	"	.0127		.0064		1.98
7	"	.0365		.0117		3.12
7	"	.0276		.0079		3.49
7	"	.0409		.0198		2.06
7	"	.0459		.0189		2.43
7	"	.0291		.0123		2.36
			.0308		.0122	
Mean Value of Above teeth		.0308		.0118		2.89

TABLE II

Deciduous Teeth - 7.3 years exposure (under 6 years)

Age (Yrs)	Type of Tooth	% Fluorine in Dentin	Age Mean	% Fluorine in Enamel	Age Mean	D/E
5	Molar	.0478		.0186		2.57
5	"	.0311		.0053		6.39
5	"	.0245		.0090		3.06
			.0311		.0099	
6	"	.0345		.0185		2.54
6	"	.0354		.0115		2.90
6	"	.0282		.0109		2.31
			.0310		.0126	
7	Molar	.0228		.0084		2.71
7	"	.0127		.0064		1.98
7	"	.0365		.0117		3.12
7	"	.0276		.0079		3.49
7	"	.0409		.0198		2.06
7	"	.0459		.0189		2.43
7	"	.0291		.0125		2.36
			.0308		.0128	
Mean Value of Above teeth		.0308		.0118		2.89

TABLE III

Deciduous Teeth - 7.5 years exposure (8 years and over)

Age (yrs)	Type of Tooth	% Fluorine in Dentin	Age Mean	% Fluorine in Enamel	Age Mean	D/E
8	Molar	.0328		.0073		4.49
8	"	.0199		.0044		4.52
8	"	.0065		.0071		0.92
8	Incisor	.0298		.0070		4.26
8	Molar	.0278		.0066		4.21
8	Cuspid	.0368		.0208		1.77
8	Incisor	.0336		.0058		5.79
8	Molar	.0251		.0035		7.17
8	Molar	.0306		.0049		6.22
			.0270		.0075	
9	Molar	.0426		.0087		4.90
9	"	.0258		.0033		7.81
9	"	.0271		.0173		1.56
9	"	.0263		.0059		4.45
9	Cuspid	.0239		.0018		13.27
			.0291		.0074	
10	Cuspid	.0150		.0054		2.77
10	Molar	.0272		.0277		1.00
10	Cuspid	.0195		.0067		2.91
			.0206		.0133	
11	Molar	.0473		.0095		4.98
11	"	.0118		.0033		3.57
11	"		.0296		.0064	
12	Molar	.0227		.0034		6.68
12	Cuspid	.0150		.0015		10.00
			.0189		.0024	
13	Cuspid	.0159		.0045		3.53
			.0159		.0045	
Mean Value of Above teeth		.0256		.0076		4.85

TABLE III

Deciduous Teeth - 7.5 years exposure (8 years and over)

Age (yrs)	Type of Tooth	% Fluorine in Dentin	Age Mean	% Fluorine in Enamel	Age Mean	D/E
8	Molar	.0258		.0073		4.49
8	"	.0159		.0044		4.32
8	"	.0065		.0071		0.92
8	Incisor	.0298		.0070		4.26
8	Molar	.0278		.0066		4.21
8	Cuspid	.0268		.0038		1.77
8	Incisor	.0256		.0038		5.79
8	Molar	.0281		.0035		7.17
8	Molar	.0306		.0049		6.22
			.0270		.0075	
9	Molar	.0428		.0087		4.90
9	"	.0258		.0038		7.81
9	"	.0271		.0175		1.56
9	"	.0265		.0059		4.45
9	Cuspid	.0238		.0018		13.27
			.0291		.0074	
10	Cuspid	.0150		.0054		2.77
10	Molar	.0272		.0277		1.00
10	Cuspid	.0195		.0087		2.91
			.0206		.0135	
11	Molar	.0475		.0095		4.98
11	"	.0118		.0073		3.27
11	"		.0296		.0084	
12	Molar	.0287		.0034		6.68
12	Cuspid	.0150		.0015		10.00
			.0189		.0024	
13	Cuspid	.0159		.0045		3.53
			.0159		.0045	

Mean Value of
Above teeth .0256 .0076 4.85

TABLE IV

PERMANENT TEETH -- 3.0 years exposure

Age (yrs)	Type of Tooth	%Fluorine in Dentin	Age Mean	% Fluorine in Enamel	Age Mean	D/E
8	Molar	.0193		.0179		1.1
8	"	.0086		.0097		0.9
8	"	.0173		.0052		3.3
			.0151		.0109	
10	Molar	.0350		.0324		1.1
			.0350		.0324	
12	Molar	.0115		.0042		2.6
12	"	.0181		.0040		4.4
12	"	.0116		.0049		2.4
12	Bicuspid	.0105		.0078		1.3
12	"	.0228		.0074		3.1
			.0149		.0057	
13	Molar	.0167		.0032		5.2
13	"	.0124		.0062		2.0
			.0145		.0047	
15	Molar	.0114		.0029		3.9
15	"	.0136		.0036		3.8
15	"	.0201		.0049		4.1
			.0150		.0038	
17	Bicuspid	.0221		.0123		1.8
			.0221		.0123	
Mean Value of Above teeth		,0167		.0084		2.7

TABLE IV

PERMANENT TEETH -- 5.0 years exposure

Age (yrs)	Type of Tooth	% Fluorine in Dentin	Age Mean	% Fluorine in Enamel	Age Mean	D/E
8	Molar	.0195		.0179		1.1
8	"	.0086		.0097		0.9
8	"	.0173		.0052		3.3
			.0151		.0109	
10	Molar	.0350		.0254		1.1
			.0350		.0254	
12	Molar	.0115		.0042		2.6
12	"	.0181		.0040		4.4
12	"	.0116		.0049		2.4
12	Bicuspid	.0105		.0078		1.3
12	"	.0228		.0074		3.1
			.0149		.0057	
13	Molar	.0187		.0052		3.2
13	"	.0184		.0052		2.0
			.0145		.0047	
15	Molar	.0114		.0029		3.9
15	"	.0136		.0036		3.3
15	"	.0201		.0049		4.1
			.0150		.0038	
17	Bicuspid	.0221		.0123		1.8
			.0221		.0123	
Mean Value of Above teeth		.0187		.0064		2.7

TABLE V.

Permanent Teeth - 7.5 years exposure

Age (yrs)	Type of Tooth	% Fluorine in Dentin	Age Mean	% Fluorine in Enamel	Age Mean	D/E
9	Bicuspid	.0267		.0093		2.87
9	Molar	.0487		.0182		2.68
9	"	.0468		.0214		2.19
9	"	.0474		.0178		2.66
9	"	.0290		.0104		2.79
9	Bicuspid	.0315		.0094		3.35
			.0383		.0144	
10	Incisor	.0262		.0050		5.24
			.0262		.0050	
11	Molar	.0348		.0038		9.16
11	Bicuspid	.0357		.0138		2.59
11	Incisor	.0279		.0054		5.17
11	Bicuspid	.0383		.0096		3.99
11	"	.0891		.0265		3.36
11	"	.0338		.0130		2.60
11	Molar	.0339		.0341		0.99
11	Bicuspid	.0480		.0128		3.75
11	"	.0182		.0101		1.80
11	"	.0321		.0070		4.59
			.0392		.0136	
12	Molar	.0150		.0044		5.93
			.0150		.0044	
13	Molar	.0395		.0080		4.94
13	"	.0390		.0073		5.34
13	Bicuspid	.0396		.0116		3.41
13	"	.0384		.0110		3.49
			.0391		.0095	
14	Molar	.0165		.0078		2.12
14	"	.0486		.0130		3.74
14	"	.0335		.0578		0.58
14	"	.0229		.0079		2.89
14	Bicuspid	.0319		.0063		5.06
			.0311		.0088*	
15	Molar	.0225		.0235		0.96
15	Bicuspid	.0439		--		--
15	"	.0479		.0118		4.06
15	Molar	.0360		.0068		5.29
15	"	.0188		.0059		3.19
			.0338		.0120	
16	Molar	.0406		.0116		3.50
			.0406		.0116	
17	Molar	.0518		.0083		6.24
			.0518		.0083	
Mean Value of Above Teeth		.0363		.0130		3.65

* omitting the high figure .0578.

TABLE V.

Permanent Teeth - 7.5 years exposure

Age (yrs)	Type of Tooth	% Fluorine in Dentin	Age Mean	% Fluorine in Enamel	Age Mean	D/E
9	Bicuspid	.0287		.0093		3.87
9	Molar	.0487		.0182		2.68
9	"	.0488		.0214		2.19
9	"	.0474		.0178		2.66
9	"	.0290		.0104		2.79
9	Bicuspid	.0315		.0094		3.35
			.0385		.0144	
10	Incisor	.0262		.0050		5.24
			.0262		.0050	
11	Molar	.0548		.0058		9.16
11	Bicuspid	.0237		.0138		2.39
11	Incisor	.0279		.0054		5.17
11	Bicuspid	.0388		.0096		3.99
11	"	.0891		.0265		3.76
11	"	.0238		.0130		2.80
11	Molar	.0339		.0341		0.99
11	Bicuspid	.0480		.0128		3.75
11	"	.0182		.0101		1.80
11	"	.0321		.0070		4.59
			.0398		.0136	
12	Molar	.0180		.0044		5.32
			.0180		.0044	
13	Molar	.0395		.0090		4.94
13	"	.0290		.0075		3.74
13	Bicuspid	.0396		.0116		3.41
13	"	.0394		.0110		3.49
			.0391		.0095	
14	Molar	.0165		.0078		2.12
14	"	.0486		.0130		3.74
14	"	.0388		.0078		0.39
14	"	.0229		.0079		2.89
14	Bicuspid	.0319		.0065		5.06
			.0311		.0088*	
15	Molar	.0229		.0235		0.96
15	Bicuspid	.0439		--		--
15	"	.0479		.0118		4.06
15	Molar	.0380		.0068		5.29
15	"	.0188		.0059		3.19
			.0338		.0120	
16	Molar	.0406		.0116		3.50
			.0406		.0116	
17	Molar	.0318		.0083		6.24
			.0318		.0083	
Mean Value of Above Teeth		.0262		.0130		3.68

* omitting the high figure .0378.

FIGURE I

Rate of Accumulation of Fluorine in Permanent Teeth

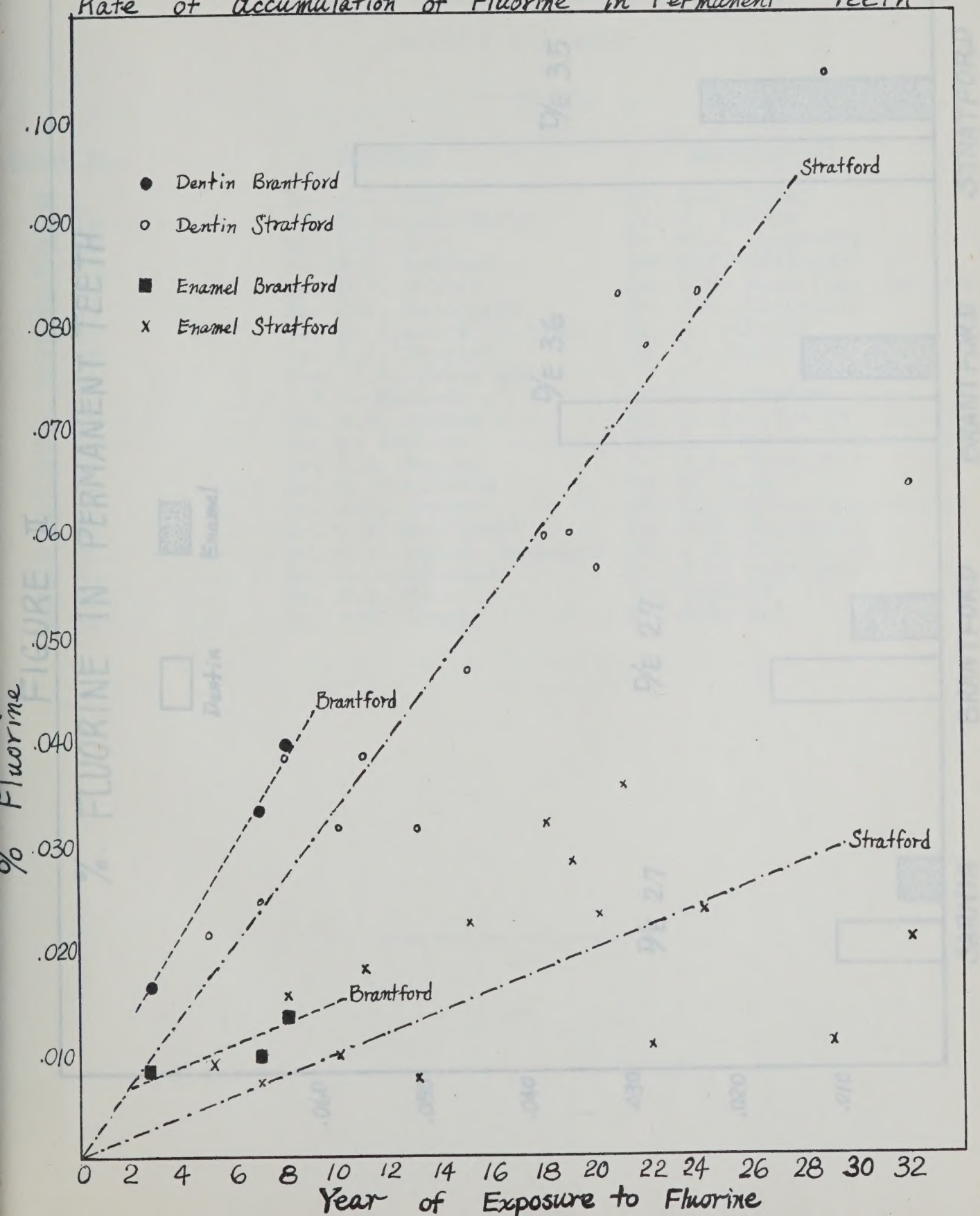
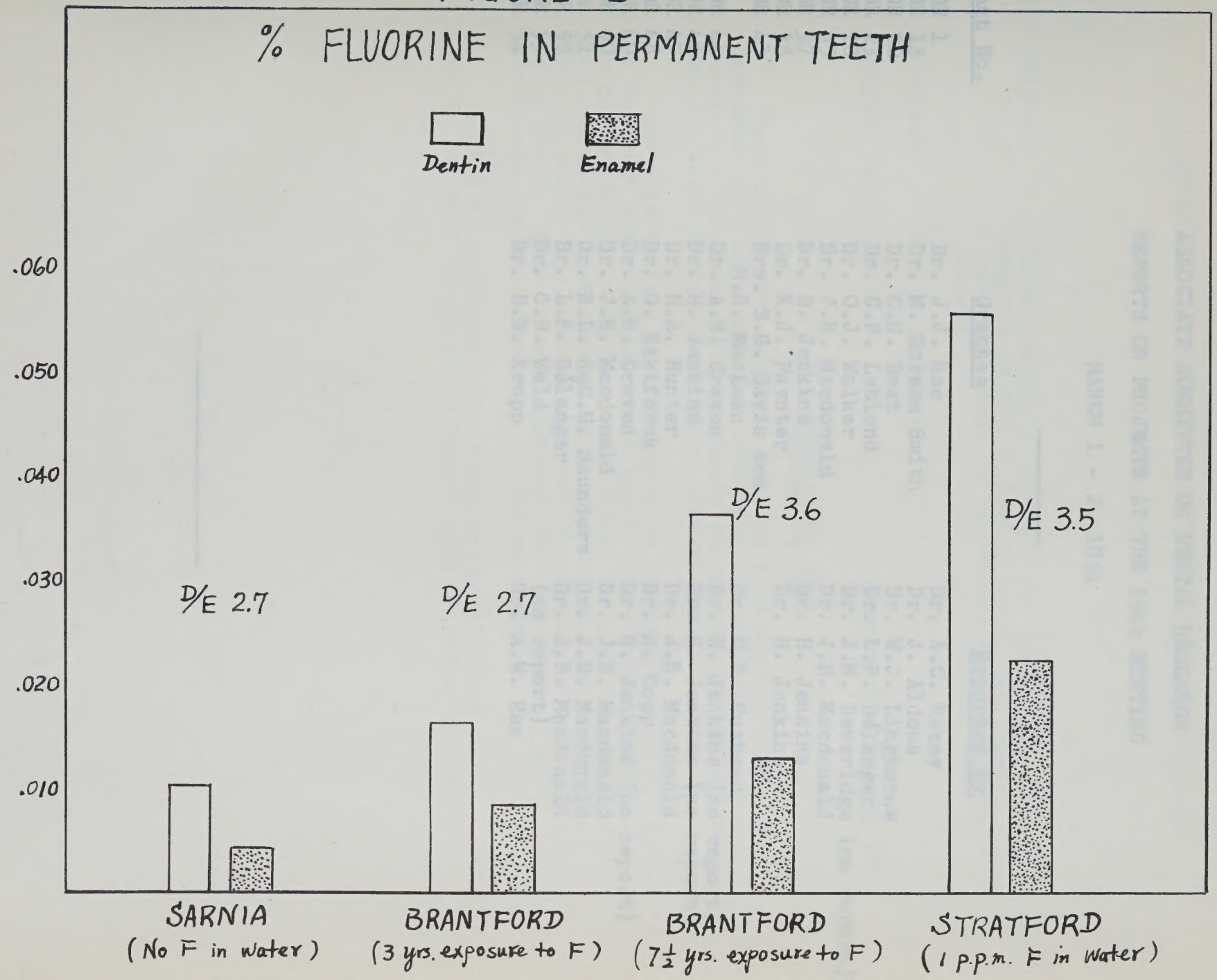


FIGURE I

FIGURE II


% FLUORINE IN PERMANENT TEETH
Dentin
Enamel
.060
.050
.040
.030
.020
.010
D/E 2.7
D/E 2.7
D/E 3.6
D/E 3.5
SARNIA
(No F in water)
BRANTFORD
(3 yrs. exposure to F)
BRANTFORD
(7½ yrs. exposure to F)
STRATFORD
(1 p.p.m. F in water)

% FLUORINE IN PERMANENT TEETH
Enamel
Dentin
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
SWANSEA
BRANTFORD
STRATFORD

APPENDIX "R"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

REPORTS ON PROJECTS AT THE 19th MEETING

MARCH 1 - 2, 1954

Grant No.	Grantee	Reported by
DR 1	Dr. J.J. Rae	Dr. A.C. Racey
DR 13	Dr. M. Doreen Smith	Dr. J. Aldous
DR 22	Dr. C.H. Best	Dr. W.J. Linghorne
DR 23	Dr. C.P. Leblond	Dr. L.F. Bélanger
DR 28	Dr. O.J. Walker	Dr. J.M. Beveridge (no report)
DR 35	Dr. J.B. Macdonald	Dr. J.B. Macdonald
DR 37	Dr. H. Jenkins	Dr. H. Jenkins
DR 38	Dr. K.J. Paynter	Dr. H. Jenkins
DR 43	Drs. S.G. Davis and H.R. MacLean	Dr. C.D. Husband
DR 44	Dr. A.H. Craven	Dr. H. Jenkins (no report)
DR 46	Dr. H. Jenkins	Dr. H. Jenkins (no report)
DR 47	Dr. H.A. Hunter	Dr. J.B. Macdonald
DR 48	Dr. G. Nikiforuk	Dr. H. Copp
DR 49	Dr. A.H. Craven	Dr. H. Jenkins (no report)
DR 50	Dr. J.B. Macdonald	Dr. J.B. Macdonald
DR 51	Dr. R.L. deC.H. Saunders	Dr. J.B. Macdonald
DR 52	Dr. L.F. Bélanger	Dr. J.B. Macdonald
DR 53	Dr. C.B. Weld	(no report)
DR 54	Dr. B.N. Kropp	Dr. A.W. Ham

"APPENDIX "B"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

REPORTS ON PROJECTS AT THE 19th MEETING

MARCH 1 - 2, 1954

Grant No.	Grantee	Reported by
DR 1	Dr. J.J. Rae	Dr. A.C. Racey
DR 13	Dr. M. Doreen Smith	Dr. J. Aldous
DR 22	Dr. G.H. Best	Dr. W.J. Linghorne
DR 23	Dr. O.P. Lebland	Dr. L.F. Bélanger
DR 28	Dr. O.J. Walker	Dr. J.M. Beveridge (no report)
DR 35	Dr. J.B. Macdonald	Dr. J.B. Macdonald
DR 37	Dr. H. Jenkins	Dr. H. Jenkins
DR 38	Dr. K.J. Paynter	Dr. H. Jenkins
DR 43	Drs. S.G. Davis and H.R. Macleam	Dr. C.D. Husband
DR 44	Dr. A.H. Craven	Dr. H. Jenkins (no report)
DR 46	Dr. H. Jenkins	Dr. H. Jenkins (no report)
DR 47	Dr. R.A. Hunter	Dr. J.B. Macdonald
DR 48	Dr. G. Nikiforuk	Dr. H. Copp
DR 49	Dr. A.H. Craven	Dr. H. Jenkins (no report)
DR 50	Dr. J.B. Macdonald	Dr. J.B. Macdonald
DR 51	Dr. R.L. deG.H. Saunders	Dr. J.B. Macdonald
DR 52	Dr. L.F. Bélanger	Dr. J.B. Macdonald
DR 53	Dr. C.B. Weld	(no report)
DR 54	Dr. B.N. Kropp	Dr. A.V. Ham

APPENDIX "S"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1954

Subject: Investigation of methods for destroying the organic matter in teeth, bones, and other materials prior to determination of fluorine content

Grantee: Dr. O.J. Walker

Project No.: DR 28 Amount of Grant: $1000

SUMMARY OF WORK

Very little work has been done on this project since September 1953 as a research assistant has not been available. However, I have been able to obtain the services of Miss Hinda Doz for eight hours a week since January 1st.

Our efforts have been directed to clearing up the matter of the high blanks mentioned in the Progress Report submitted in September 1953. We have established the fact that they are not due to the distillation over of sulfates. At the present time we are of the opinion that the Photoelectric Colorimeter is at fault. This is an instrument that has been in use constantly for ten years with little maintenance.

Work will continue on this project until early in April. At that time a final report will be prepared and sent to you.

APPENDIX "T"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

(i) List of Grantees and Subjects

rant No.		Grantee	Subject
DR 1	Active	Dr. J.J. Rae, Dept. of Chemistry, University of Toronto	Chemical mechanisms in dental caries
DR 2	Completed	Dr. G.H.W. Lucas, Dept. of Pharmacology, University of Toronto	A study of alveolar oxygen tension and blood oxygen content during nitrous oxide anaesthesia
DR 3	Completed	Dr. H.K. Box, Faculty of Dentistry, University of Toronto	A study of micro-organisms found in deep periodontal pockets and caries lesion as revealed by the electro-microscope
DR 4	Completed	Dr. H.K. Box, Faculty of Dentistry, University of Toronto	An investigation of packing material in the treatment of periodontal disease (continuation of investigation previously carried on by Maj. Linghorne)
DR 5	Completed	Major R.L. Twible, Royal Canadian Dental Corps (later Ontario Research Foundation)	To improve certain properties of the acrylic resin denture base and to effect an economy in denture production
DR 6	Completed	Brig. E.M. Wansbrough, Royal Canadian Dental Corps	To study the effects of altitude acceleration and deceleration on oral tissues
DR 7	Completed	Dr. J.S. Bagnall, Faculty of Dentistry, Dalhousie University	Critical survey of the literature on dental caries
DR 8	Completed	Dr. A.D.A. Mason, Faculty of Dentistry, University of Toronto	A study of the D.M.F. caries rate of the children of Brantford prior to the addition of fluorine to the water supply

Grant No.		Grantee	Subject
DR 9	Completed	Dr. O.J. Walker, Dept. of Chemistry, Dr. H.R. MacLean, Faculty of Dentistry, University of Alberta	An investigation of the effects of certain chemicals on material in appliances used in prosthetic dentistry
DR 10	Completed	Dr. G.M. Macdonald, Queen Mary Veterans' Hospital, Montreal	Facial prostheses
DR 11	Completed	Dr. R.J. Godfrey, Faculty of Dentistry, University of Toronto	An anatomical, histological and physiological study of the role of the Condyle in Mastication
DR 12	Completed	Dr. A.E.R. Westman, Ontario Research Foundation	Investigation of cements for methacrylate inlays
DR 13	Active	Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Fluoride content of dentine, enamel and alveolar processes of teeth from Sarnia, Brantford and Stratfo
DR 14	Completed	Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Fluorine analysis of wat and food samples from different areas in Ontario collected and studied in relation t incidence of dental caries
DR 15	Completed	Dr. Norma Ford Walker, Dept. of Zoology, University of Toronto	A comparison of the patterns of eruption the deciduous teeth i man with rate of grow of the dental arches
DR 16	Completed	Dr. C.H. Best, Dept. of Medical Research, University of Toronto	Studies in vitro of certain fungus-like organisms found in periodontal diseases
DR 17	Completed	Dr. R.F. Shaner, Dept. of Anatomy, University of Alberta	An investigation of the effects of solutions used in operative dentistry on the vita pulps of teeth

rant No.		Grantee	Subject
DR 18	Completed	Dr. H.K. Box, Faculty of Dentistry, University of Toronto	Experimental production of dental caries
DR 19	Completed	Dr. E.A. Sellers, Dept. of Pharmacology, University of Toronto	Tissue toxicity and efficiency of local anaesthetics for topical application and nerve block in dentistry
DR 20	Completed	Dr. Jules Thebaud, Faculty of Dental Surgery, University of Montreal	Improvement of subgingival curettage and surgical techniques used in the treatment of pyorrhea alveolaris
DR 21	Completed	Dr. A.E.R. Westman, Ontario Research Foundation	Investigation of repair techniques for methacrylate dentures
DR 22	Active	Dr. C.H. Best, Dept. of Medical Research, University of Toronto	An investigation of the mechanism of repair of the supporting structures of the teeth and factors affecting the reparative process
DR 23	Active	Dr. C.P. Leblond, Dept. of Anatomy, McGill University	Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radioactive elements
DR 24	Completed	Dr. C.H.M. Williams, Faculty of Dentistry, University of Toronto	The effects of hard and soft diets on the supporting structures of the teeth
DR 25	Completed	Dr. A.E.R. Westman, Ontario Research Foundation	Investigation of methods for the use of methyl methacrylate in direct dental fillings
DR 26	Completed	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	A study of the normal and abnormal physiologic forces of the oral cavity
DR 27	Completed	Dr. J.W. Abbiss, Faculty of Dentistry, Dr. A.G. Nutlay, Faculty of Dentistry, Dalhousie University	A histological study of tooth germ development, both intra and extra alveolar, with a survey of pathological changes in the developing tooth

Grant No.		Grantee	Subject
DR 28	Active	Dr. O.J. Walker, Dept. of Chemistry, University of Alberta	Investigation of methods for destroying the organic matter in tee bones and other mater ial prior to determin ation of fluorine con tent
DR 29	Completed	Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Biological absorption of fluorine
DR 30	Completed	Dr. Norma Ford Walker, Dept. of Zoology, University of Toronto	The study of the closure of space following pr mature loss of decidu ous teeth
DR 31	Completed	Dr. L.B. Jaques, Dept. of Physiology, Mr. G.J. Millar, Dept. of Physiology, University of Saskatchewan	An investigation of nerve block
DR 32	Completed	Dr. A.W. Ham, Dept. of Anatomy, University of Toronto	Investigation of the effect of adrenal cor tical hormone imbalan ces on the various connective tissue structures of the gro ing incisor teeth of animals
DR 33	Completed	Dr. H.A. Hunter, Faculty of Dentistry, University of Toronto	A study of calcifying potential of calcium oleate on the dental pulp
DR 34	Completed	Dr. A.D.'Iorio, Dept. of Physiology, University of Montreal	Studies of calcium metabolism in tooth with the aid of Ca^{45}
DR 35	Active	Dr. J.B. Macdonald, Dept. of Bacteriology, University of Toronto	Bacteriology of periodon tal disease I. Experi mental fusospirocheta infection with mixtur of pure culture
DR 36	Completed	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	Demineralization of hard tissues as bone and teeth by organic che- lating agents

ant No.		Grantee	Subject
DR 37	Active	Dr. H. Jenkins, Dept. of Orthodontics, University of Toronto	Further studies in the electromyography of the head, face and neck
DR 38	Active	Dr. K.J. Paynter, Faculty of Dentistry, University of Toronto	The effects of the endocrines on the teeth and jaws. II. Further studies on the effect of thiouracil on the development of molar teeth of rats
DR 39	Completed	Dr. A.G. Racey, Faculty of Dentistry, McGill University	Sectioning of teeth and surrounding structures and the effects of potassium deficiency and of pyriodoxine deficiency on the teeth and surrounding structures of dogs
DR 40	Completed	Dr. L.A. Funt, Faculty of Dentistry, University of Toronto	Effect of tooth eruption and function on the growing of the mandible and facial complex on cats
DR 41	Completed	Dr. Harvey Jenkins, Faculty of Dentistry, University of Toronto	A study of the dental arch form and certain of its anatomical relations in a series of clinically excellent dentitions (After Downs (1))
DR 42	Completed	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	Studies in the chemistry of the saliva. II. The reducing property of saliva towards some oxidation-reduction indicators
DR 43	Active	Dr. S.G. Davis, Dept. of Chemistry, Dr. H.R. MacLean, Faculty of Dentistry, University of Alberta	Electro potentials caused by dental alloys
DR 44	Completed	Dr. Arthur Harry Craven, Faculty of Dentistry, McGill University	Facial and cranial growth patterns in the rat as revealed by vital staining with alizarine red S

Grant No.		Grantee	Subject
DR 45	Completed	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	Study of the incidence of malocclusion
DR 46	Active	Dr. H. Jenkins, Dept. of Orthodontics, University of Toronto	Study of treated cases
DR 47	Active	Dr. H.A. Hunter, Faculty of Dentistry, University of Toronto	The pathological characteristics of experimental fusospirocheta infections
DR 48	Active	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	The effect of topical application of silicon in protecting teeth against acid solution in vitro and dental caries produced in vitro
DR 49	Completed	Dr. A.H. Craven, Faculty of Dentistry, University of Toronto	A recording device to determine postural changes of the head i the horizontal plane
DR 50	Active	Dr. J.B. Macdonald, Dept. of Bacteriology, University of Toronto	The cultivation and characteristics of bacteroides nigrescen
DR 51	Active	Dr. R.L. deC.H. Saunders, Prof. of Anatomy, Dalhousie University	Study of human dental pulp blood vessels by X-ray micro-arteriographic methods
DR 52	Active	Dr. L.F. Belanger, Dept. of Histology and Embryology, University of Ottawa	Mechanism of bone and tooth formation in th light of autoradiography and histochemistry
DR 53	Completed	Dr. C.B. Weld, Dept. of Physiology, Dalhousie University	The macrophages in the pulp tissue of the ra incisor and lower jaw
DR 54	Active	Dr. B.N. Kropp, Faculty of Medicine, Queen's University	Development of a rapid method for grinding thin sections of plastic-embedded teet

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

(ii) Summary of Grants by Year 1945-55

Grant	Grantee	1945-46 $	46-47 $	47-48 $	48-49 $	49-50 $	50-51 $	51-52 $	52-53 $	53-54 $	54-55 $
DR 1	Rae	-	900	1000 250	1760 150	1000 550	950	960	1530	1680	1080
DR 2	Lucas	1550	2950	1900	1200 400	325	1550	-	-	-	-
DR 3	Box	1000	500	500	300	100	-	-	-	-	-
DR 4	Linghorne R.C.D.C.	-	(Box) 2200 1000	2200	-	-	-	-	-	-	-
DR 5	Twible R.C.D.C.	-	(Ont.Res. Foundation) 4800	-	-	-	-	-	-	-	-
DR 6	Coons R.C.D.C.	-	Wansbrough	-	-	-	-	-	-	-	-
DR 7	Bagnall	200	300	225	-	75	-	-	-	-	-
DR 8	Ellis (Mason)	-	1000	completed	-	-	-	-	-	-	-
DR 9	Walker MacLean	-	1000	1000	1100	300	-	-	-	-	-
DR 10	Macdonald	-	800	completed	-	-	-	-	-	-	-
DR 11	Godfrey	-	1425 (half-year)	2550	2200	559.07	-	-	-	-	-
DR 12	Westman	-	-	2550	See DR 21	See DR 25	See DR 25	See DR 25			-

Grant	Grantee	1945-46 $	46-47 $	47-48 $	48-49 $	49-50 $	50-51 $	51-52 $	52-53 $	53-54 $	54-55 $
DR 13	Smith	-	-	1050	1455	1475	1475	1600	500	1625	1625
DR 14	Smith	-	-	1125	1500	-	-	-	-	-	-
DR 15	Walker, N.F.	-	-	3000	3000	2500	800	-	-	-	-
DR 16	Best	-	-	1100	1500	-	-	-	-	-	-
DR 17	Shaner	-	-	1000	-	-	-	-	-	-	-
DR 18	Box	-	-	1350	1620	1620 458.80	-	-	-	-	-
DR 19	Sellers	-	-	1300	1575	Ferguson 320	-	-	-	-	-
DR 20	Thebaud	-	-	800	750	750	-	-	-	-	-
DR 21	Westman	-	-	-	5000	-	-	-	-	-	-
DR 22	Box (later Best)	-	-	-	2200	2400	3800	2300 875 1350	4200	4200	4200
DR 23	Leblond	-	-	-	2200	2200 400	2400	2400	3000	3000	3500
DR 24	Williams	-	-	-	1420	3080	1735	-	-	-	-
DR 25	Westman (Ont.Res. Foundation)	-	-	-	-	4000	3000	4000	-	-	-
DR 26	Moyers	-	-	-	-	1300	150	-	-	-	-
DR 27	Abbiss Nutlay	-	-	-	-	1000	200	-	-	-	-

Grant	Grantee	1945-46 $	46-47 $	47-48 $	48-49 $	49-50 $	50-51 $	51-52 $	52-53 $	53-54 $	54-55 $
DR 28	Walker, O.J.-MacLean	-	-	-	-	1000	1000	1000	600	1000	-
DR 29	Smith	-	-	-	-	1500	375	-	-	-	-
DR 30	Walker, N.F.	-	-	-	-	1200 600	1400 1200	-	-	-	-
DR 31	Jaques Millar	-	-	-	-	-	1870	188	-	-	-
DR 32	Ham	-	-	-	-	-	900	-	-	-	-
DR 33	Hunter	-	-	-	-	-	1500	1600	-	-	-
DR 34	D'Iorio	-	-	-	-	-	2840	1200 (Lussier)	-	-	-
DR 35	Macdonald	-	-	-	-	-	2000	2800	3300	3610	2300
DR 36	Nikiforuk	-	-	-	-	-	-	2400 350	1600	-	-
DR 37	Jenkins	-	-	-	-	-	-	960	800	300	225
DR 38	Paynter	-	-	-	-	-	-	1850	2610	1000	-
DR 39	Racey	-	-	-	-	-	-	1600	1600	-	-
DR 40	Funt Sayers	-	-	-	-	-	-	325	-	-	-
DR 41	Jenkins	-	-	-	-	-	-	300	-	-	-
DR 42	Nikiforuk	-	-	-	-	-	-	-	1500	-	-

Grant	Grantee	1945-46 $	46-47 $	47-48 $	48-49 $	49-50 $	50-51 $	51-52 $	52-53 $	53-54 $	54-55 $
DR 43	Davis MacLean	-	-	-	-	-	-	-	150	1025	250
DR 44	Craven	-	-	-	-	-	-	-	600	1350	-
DR 45	Moyers	-	-	-	-	-	-	-	1700	-	-
DR 46	Moyers	-	-	-	-	-	-	-	520	520	-
DR 47	Hunter	-	-	-	-	-	-	-	1200	2200	2620
DR 48	Nikiforuk	-	-	-	-	-	-	-	-	2240	-
DR 49	Craven	-	-	-	-	-	-	-	-	400	-
DR 50	Macdonald	-	-	-	-	-	-	-	-	400	400
DR 51	Saunders	-	-	-	-	-	-	-	-	1200	1870
DR 52	Belanger	-	-	-	-	-	-	-	-	1340	3337.75
DR 53	Weld	-	-	-	-	-	-	-	-	2985	-
DR 54	Kropp	-	-	-	-	-	-	-	-	820	650

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6.	Dr. R.H. McDougall, 1505 Hampshire Road, Victoria, B.C.	31 March, 1955
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	Representing the Division of Dental Health Department of National Health and Welfare	
9.	Dr. H.K. Brown, ex officio, Chief, Dental Health Division, Dept. of National Health and Welfare, Ottawa, Ont.	--

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*8.	Brig. E.M. Wansbrough, ex officio, -- Director General of Dental Services, Royal Canadian Dental Corps, Dept. of National Defence, Ottawa, Ont.	
	Representing the Division of Dental Health Department of National Health and Welfare	
9.	Dr. H.K. Brown, ex officio, -- Chief, Dental Health Division, Dept. of National Health and Welfare, Ottawa, Ont.	

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*15. Dr. A.W. Ham, Professor of Anatomy, Faculty of Medicine, University of Toronto, Toronto, Ont. 31 March, 1955

16. Dr. J.D. Hamilton, Professor of Pathology, University of Toronto, Toronto, Ont. 31 March, 1954

17. Dr. J.B. Macdonald, Assistant Professor of Bacteriology, Faculty of Dentistry, University of Toronto, Toronto, Ont. 31 March, 1954

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*19. Mr. S.J. Cook, Secretary, Public Relations Branch, National Research Council, Ottawa, Ont. --

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